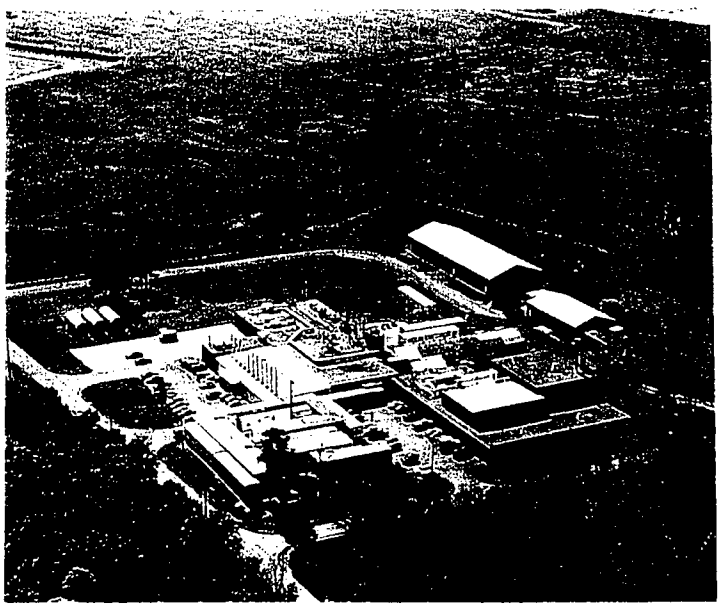
REPORT

Attachments D – M

FINAL REPORT

Task 94-33: *In Vivo* Evaluation of Temporary Wound Dressings for Adherence, Durability and Autografting on Sulfur Mustard-Induced Lesions in Weanling Swine



To

U.S. Army Medical Research Institute of Chemical Defense

December, 2000

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ATTACHMENT D

Phase I Statistics Reports for Phase I, Part B

Project Number G1555-B33ASTAT (3104)

Battelle
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Date April 16, 1998

To Frances Reid

From Nancy Niemuth

Subject **Additional Analysis of Task 33, Phase 1B Data**

Internal Distribution

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Additional Analysis Report.wpd

The attached statistical report summarizes additional analysis of serum chemistry, hematology, and thiodiglycol data collected in Phase 1B of MREF Task 94-33.

NN:kc

Attachment

For Review and Approval

	Name	Initials	Date
Originator	N. Niemuth	N	4/16/98
Concurrence	R. Menton	RMZ	4/16/98
Approved	J. Orban	BS	4/17/98

MREF Task 94-33, Phase 1B
Statistical Analysis of Serum Chemistry, Hematology, and Thiodiglycol Data

Introduction

This report summarizes the statistical analysis of serum chemistry, hematology, and thiodiglycol data collected in Phase 1B of MREF Task 94-33. This report should be considered a supplement to the Phase 1B statistical report dated February 5, 1998. The conclusions drawn in the previous report remain valid.

Statistical Methods

Serum chemistry and hematology data for each animal on study days 0, 1, 3, and 7 were included in the statistical analysis. Twice daily thiodiglycol readings from study days 0 - 7 were included in the analysis. For each parameter, the following analysis of variance (ANOVA) model was fitted to the data:

$$y_{ij}=\mu+\alpha_i+\tau_j+\epsilon_{ij}$$

where y_{ij} is the reading for animal j on study day i, μ is the overall mean, α_i is a fixed effect for study day i, τ_j is a random effect for animal j, and ϵ_{ij} is a random error term. Appropriate contrasts were used to estimate the difference in means between study days. For the serum chemistry and hematology parameters, all pairwise comparisons between study day means were estimated. For the thiodiglycol parameters, AM and PM means on each study day were compared to the pretreatment mean (study day 0, AM reading). For each parameter, a Bonferroni adjustment for multiple comparisons was applied to ensure that the probability of making at least one incorrect conclusion of significance is no higher than 0.05. The SAS (V6.12) MIXED procedure was used to fit the statistical models.

Results

Descriptive statistics and statistical comparisons are presented in Tables 1, 2, and 3, for the serum chemistry, hematology, and thiodiglycol parameters, respectively. Figures A-1 through A-38 present the means and 95 percent confidence intervals for each parameter, in the order they are presented in Tables 1 to 3. Similarly, Figures B-1 through B-38 present the data for each animal.

Statistical comparisons of serum chemistry and hematology data are summarized by the letters entered in the last row for each parameter in Tables 1 and 2. For these comparisons, study day means that were not significantly different (at an over all 0.05 level) share at least one letter and those that were significantly different do not have a common letter. When no significant differences were noted among the study days, the single letter A appears in each cell (see Aspartate Transaminase). For parameters where significant differences were noted, the results are summarized below, in addition to the table.

Serum Chemistry Parameters (Table 1)

Alanine Transaminase: Means on days 1 and 3 were greater than that on day 7. No differences were noted in comparisons to the mean on day 0.

Albumin: The mean on day 3 was greater than that on day 7.

Alkaline Phosphatase: The mean on day 0 was greater than on days 1, 3, and 7. The mean on day 1 was greater than on days 3 and 7. The means on days 3 and 7 were not significantly different.

Amylase: The mean on day 0 was greater than on days 1, 3, and 7.

Blood Urea Nitrogen: The mean on day 0 was less than on days 1, 3, and 7.

Calcium : The mean on day 1 was significantly less than that on day 7.

Chloride: The mean on day 3 was greater than those on days 0 and 7, but not significantly different from that on day 1.

Globulin: The mean on day 7 was greater than on days 0, 1, and 3.

Phosphorus: The mean on day 3 was less than those on days 0 and 7, but not significantly different from that on day 1.

Ratio of Blood Urea Nitrogen to Creatinine: The mean on day 7 was greater than that on day 0.

Ratio of Albumin to Globulin: The mean on day 7 was less than on days 0, 1, and 3.

Hematology Parameters (Table 2)

Basophils: The mean on day 7 was greater than on days 0 and 1, but not significantly different from that on day 3. Also, the mean on day 3 was greater than that on day 1.

Eosinophils: The mean on day 7 was greater than on days 0 and 1, but not significantly different from that on day 3. Also, the mean on day 3 was greater than that on day 1.

Mean Corpuscular Hemoglobin: The mean on day 7 was less than on day 1.

Mean Corpuscular Concentration: The mean on day 7 was less than on days 0 and 1, but not significantly different from that on day 3. Also, the mean on day 3 was less than that on day 1.

Monocytes: The means on days 0 and 1 were less than those on days 3 and 7.

Neutrophils: The mean on day 0 was less than that on day 3. The mean on day 1 was less than on days 3 and 7.

Platelet Count: The mean on day 0 was greater than that on day 3. The mean on day 7 was greater than on days 1 and 3.

White Blood Cell Count: The means on days 0 and 1 were greater than those on days 3 and 7.

Comparisons to the pretreatment mean are for thiodiglycol parameters are summarized by the letters in Table 3. In this case, the letter A in a cell indicates that the mean for that study day-time was not significantly different from the pretreatment mean. The letter B indicates the mean was different from the pretreatment mean. For thiodyglycol concentration, means on study day 0-PM and day 1-AM were significantly greater than the pretreatment (0-AM) mean. For total thiodyglycol, the mean on study day 0-PM was significantly greater than the pretreatment mean. No significant differences were noted for other study day-times. No statistical comparisons were made for urine volume.

Conclusions

The chemical burn wounds induced in Task 94-33 experiments have systemic effects, which are apparent in serum chemistry, hematology, and thiodiglycol parameters.

Table 1. Descriptive Statistics* of Serum Chemistry Parameters, by Study Day for the Six Animals Tested in Phase I, Part B.

Parameter	Study Day				
	Physical	0	1	3	7
Alanine Transaminase (U/L)	54.5 (51-58) 4.9 2	73.6 (56-97) 18.5 5 (52.6-85.3) AB	76.5 (47-103) 19.7 6 (60.5-92.5) A	74.3 (48-95) 19.4 6 (58.3-90.4) A	59.8 (42-74) 12.6 6 (43.8-75.9) B
Albumin (g/dL)	3.0 (2.6-3.3) 0.5 2	3.3 (3.0-3.5) 0.2 5 (3.1-3.5) AB	3.3 (3.1-3.7) 0.2 6 (3.1-3.5) AB	3.4 (3.1-3.8) 0.3 6 (3.2-3.6) A	3.0 (2.9-3.1) 0.1 6 (2.8-3.2) B
Alkaline Phosphatase (U/L)	450.5 (360-541) 128.0 2	343.4 (318-379) 23.9 5 (324.8-369.5) A	293.8 (243-341) 32.0 6 (272.7-315.0) B	245.0 (214-273) 19.6 6 (223.9-266.1) C	252.5 (236-284) 17.5 6 (231.4-273.6) C
Amylase (U/L)	7365.5 (6571-8160) 1123.6 2	7753.6 (4763-9739) 1960.5 5 (5902.7-9052.5) A	6299.7 (3860-8254) 1812.5 6 (4733.5-7865.8) B	5880.5 (3789-8380) 1669.5 6 (4314.4-7446.6) B	6467.3 (4431-9003) 1776.6 6 (4901.2-8033.5) B
Aspartate Transaminase (U/L)	48.0 (44-52) 5.7 2	50.2 (42-56) 5.7 5 (30.9-66.1) A	60.2 (40-79) 17.0 6 (43.9-76.4) A	44.5 (25-103) 29.1 6 (28.3-60.7) A	49.3 (40-72) 11.8 6 (33.1-65.6) A
Blood Urea Nitrogen (mg/dL)	11.6 (11.3-11.8) 0.4 2	10.0 (5.4-13.6) 3.0 5 (7.3-12.8) A	16.2 (12.9-22.4) 3.9 6 (13.7-18.7) B	16.5 (13.7-20.1) 2.3 6 (14.0-19.0) B	16.5 (14.5-18.7) 1.8 6 (14.0-19.0) B

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating significant differences (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 1. Descriptive Statistics* of Serum Chemistry Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
Calcium (mg/dL)	9.9 (9.9-9.9) 0.0 2	9.5 (9.3-9.7) 0.2 5 (9.0-9.9) AB	9.1 (8.4-9.8) 0.5 6 (8.7-9.5) A	9.7 (8.9-10.2) 0.6 6 (9.3-10.1) AB	9.9 (9.2-10.6) 0.5 6 (9.5-10.3) B
Chloride (mEq/L)	84.0 (79-89) 7.1 2	78.2 (72-88) 5.9 5 (72.6-83.1) A	82.3 (77-88) 4.5 6 (77.5-87.2) AB	87.8 (77-97) 7.4 6 (83.0-92.7) B	77.5 (73-82) 3.5 6 (72.7-82.3) A
Creatine Phosphokinase (U/L)	675.5 (485-866) 269.4 2	636.0 (371-745) 153.0 5 (-324.8-1602.5) A	915.3 (543-1882) 501.7 6 (35.6-1795.0) A	473.3 (339-702) 136.0 6 (-406.4-1353.0) A	1451.0 (407-5262) 1883.3 6 (571.3-2330.7) A
MM Isoform of Creatine Phosphokinase (%)	36.1 (29.5-42.6) 9.3 2	70.8 (55.3-91.5) 14.2 5 (61.8-83.1) A	72.5 (65.6-91.3) 9.5 6 (62.5-82.5) A	65.7 (55.9-74.4) 6.8 6 (55.8-75.7) A	71.9 (54.0-93.3) 13.7 6 (61.9-81.9) A
MB Isoform of Creatine Phosphokinase (%)	12.6 (11.7-13.4) 1.2 2	9.3 (0.0-19.6) 7.4 5 (2.9-14.7) A	6.8 (0.0-12.6) 5.4 6 (1.4-12.3) A	8.4 (0.0-14.3) 4.7 6 (2.9-13.9) A	10.1 (0.0-19.2) 7.1 6 (4.7-15.6) A
BB Isoform of Creatine Phosphokinase (%)	51.4 (44.0-58.8) 10.5 2	19.8 (8.5-26.0) 7.2 5 (11.8-27.1) A	20.7 (8.7-30.2) 7.2 6 (13.7-27.7) A	25.9 (15.3-44.0) 10.3 6 (18.8-32.9) A	18.0 (6.7-26.8) 6.7 6 (11.0-25.0) A

* MEAN
(MIN-MAX)
STD
N
(95% CI)
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Table 1. Descriptive Statistics* of Serum Chemistry Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
Creatinine (mg/dL)	0.9 (0.9-0.9) 0.0 2	0.9 (0.8-1.1) 0.1 5 (0.8-1.1) A	1.1 (0.9-1.2) 0.1 6 (0.9-1.2) A	1.1 (0.9-1.5) 0.2 6 (1.0-1.2) A	0.9 (0.8-1.0) 0.1 6 (0.8-1.0) A
Globulin (TP-ALB) (g/dL)	1.0 (0.9-1.1) 0.1 2	1.0 (0.8-1.2) 0.2 5 (0.8-1.1) A	1.0 (0.8-1.2) 0.1 6 (0.8-1.1) A	1.0 (0.8-1.3) 0.2 6 (0.8-1.1) A	1.2 (1.0-1.5) 0.2 6 (1.1-1.3) B
Glucose (Hexokinase) (mg/dL)	158.0 (153-163) 7.1 2	129.8 (98-159) 24.4 5 (110.4-161.3) A	138.0 (102-160) 25.5 6 (114.0-162.0) A	158.5 (145-191) 17.7 6 (134.5-182.5) A	150.8 (123-219) 35.3 6 (126.9-174.8) A
Lactate Dehydrogenase (U/L)	654.5 (635-674) 27.6 2	599.2 (528-637) 42.8 5 (469.8-728.6) A	597.2 (503-747) 83.7 6 (479.1-715.3) A	652.7 (536-1055) 199.6 6 (534.6-770.8) A	642.5 (528-862) 144.4 6 (524.4-760.6) A
Phosphorus (mg/dL)	9.0 (7.7-10.3) 1.8 2	10.2 (8.5-11.7) 1.2 5 (9.3-11.2) A	9.9 (8.8-11.2) 1.1 6 (9.0-10.7) AB	8.4 (7.5-9.2) 0.8 6 (7.5-9.3) B	10.4 (9.2-11.5) 0.9 6 (9.6-11.3) A
Potassium (mEq/l)	4.0 (3.8-4.2) 0.3 2	4.3 (3.9-4.8) 0.4 5 (3.2-5.4) A	3.7 (3.3-4.8) 0.6 6 (2.7-4.7) A	5.6 (3.6-8.7) 1.8 6 (4.6-6.6) A	5.1 (4.1-7.0) 1.1 6 (4.1-6.1) A

* MEAN
(MIN-MAX)
STD
N
(95% CI)
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Table 1. Descriptive Statistics* of Serum Chemistry Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
Sodium (mEq/l)	142.0 (141-143) 1.4 2	142.0 (140-145) 2.0 5 (139.4-144.8) A	141.2 (138-144) 2.0 6 (138.7-143.7) A	144.8 (138-151) 4.2 6 (142.3-147.3) A	143.3 (141-147) 2.4 6 (140.8-145.8) A
Total Protein (g/dL)	4.0 (3.5-4.4) 0.6 2	4.2 (3.8-4.7) 0.4 5 (3.9-4.5) A	4.3 (4.0-4.6) 0.2 6 (4.1-4.6) A	4.4 (3.9-4.8) 0.4 6 (4.1-4.6) A	4.2 (3.9-4.6) 0.2 6 (3.9-4.5) A
Ratio of Blood Urea Nitrogen to Creatinine	12.8 (12.6-13.1) 0.4 2	10.7 (6.8-13.6) 2.5 5 (7.4-14.1) A	15.7 (11.7-24.9) 5.0 6 (12.7-18.8) AB	15.6 (11.8-22.3) 3.6 6 (12.5-18.7) AB	18.1 (14.5-19.8) 1.8 6 (15.0-21.2) B
Ratio of Albumin to Globulin	2.9 (2.9-3.0) 0.1 2	3.4 (2.9-3.8) 0.4 5 (3.1-4.0) A	3.5 (2.7-4.1) 0.6 6 (3.0-3.9) A	3.6 (2.7-4.2) 0.6 6 (3.2-4.0) A	2.5 (2.1-3.1) 0.4 6 (2.1-3.0) B

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating significant differences (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 2. Descriptive Statistics* of Hematology Parameters, by Study Day for the Six Animals Tested in Phase I, Part B.

Parameter	Study Day				
	Physical	0	1	3	7
Basophils (#/mL)	0.048 (0.030-0.082) 0.018 6	0.071 (0.026-0.128) 0.047 5 (0.027-0.121) AB	0.058 (0.002-0.147) 0.050 6 (0.014-0.102) B	0.151 (0.096-0.261) 0.065 5 (0.103-0.197) AC	0.206 (0.161-0.269) 0.038 6 (0.162-0.249) C
Eosinophils (#/mL)	0.038 (0.005-0.078) 0.032 6	0.072 (0.009-0.134) 0.057 5 (0.004-0.139) AB	0.029 (0.006-0.090) 0.032 6 (-0.034-0.091) B	0.179 (0.031-0.277) 0.101 5 (0.105-0.240) AC	0.194 (0.077-0.292) 0.079 6 (0.132-0.257) C
Hematocrit (%)	29.850 (28.3-32.1) 1.365 6	32.060 (28.6-34.5) 2.327 5 (29.6-34.5) A	30.400 (27.5-34.5) 2.498 6 (28.1-32.7) A	31.180 (27.9-33.8) 2.519 5 (28.7-33.7) A	29.667 (24.9-32.5) 2.796 6 (27.4-31.9) A
Hemoglobin (g/dL)	10.032 (9.3-11.0) 0.587 6	10.690 (9.65-11.4) 0.714 5 (9.910-11.511) A	10.237 (9.07-11.5) 0.830 6 (9.504-10.969) A	10.146 (9.07-11.0) 0.931 5 (9.353-10.954) A	9.568 (8.13-10.3) 0.833 6 (8.836-10.301) A
Lymphocytes (#/mL)	3.350 (2.04-4.87) 1.055 6	4.338 (3.43-4.77) 0.531 5 (3.012-6.568) A	3.888 (2.21-6.53) 1.579 6 (2.194-5.583) A	5.862 (1.59-8.64) 2.623 5 (4.005-7.561) A	5.853 (3.24-8.61) 2.129 6 (4.159-7.548) A
Mean Corpuscular Hemoglobin (pg)	18.517 (17.4-19.2) 0.688 6	17.260 (16.3-18.3) 0.856 5 (16.6-18.1) AB	17.450 (16.3-18.2) 0.766 6 (16.7-18.2) A	17.320 (15.9-18.0) 0.829 5 (16.4-17.9) AB	16.667 (15.2-17.4) 0.873 6 (15.9-17.4) B

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating significant differences (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 2. Descriptive Statistics* of Hematology Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
Mean Corpuscular Concentration (g/dL)	33.617 (32.9-34.4) 0.591 6	33.340 (32.7-34.4) 0.702 5 (32.7-34.1) AB	33.650 (32.6-35.5) 1.117 6 (33.0-34.3) B	32.520 (31.5-33.2) 0.661 5 (31.8-33.2) AC	32.267 (31.7-32.6) 0.314 6 (31.6-32.9) C
Mean Corpuscular Volume (fL)	55.117 (53.1-56.5) 1.440 6	51.800 (49.6-54.4) 1.771 5 (49.9-54.1) A	51.883 (50.0-54.8) 1.716 6 (49.9-53.9) A	53.260 (49.1-55.2) 2.387 5 (50.7-54.9) A	51.783 (47.2-54.4) 2.914 6 (49.8-53.8) A
Monocytes (#/mL)	1.078 (0.788-1.37) 0.211 6	1.324 (0.712-1.94) 0.502 5 (1.013-1.723) A	1.040 (0.839-1.55) 0.309 6 (0.712-1.368) A	2.076 (1.660-2.78) 0.419 5 (1.726-2.436) B	2.503 (2.26-2.9) 0.236 6 (2.175-2.831) B
Neutrophils (#/mL)	4.803 (2.39-8.66) 2.275 6	5.284 (2.98-7.12) 1.505 5 (3.502-7.090) AB	4.370 (2.31-6.33) 1.319 6 (2.729-6.011) A	8.934 (5.81-13.40) 3.174 5 (7.092-10.679) C	8.320 (6.85-9.44) 0.929 6 (6.679-9.961) BC
Platelet Count (#/mL)	595.667 (505-697) 77.948 6	691.400 (573-846) 102.166 5 (580.7-807.1) AC	634.500 (513-862) 121.070 6 (525.7-743.3) AB	558.200 (436-714) 112.611 5 (424.1-650.5) B	785.833 (624-989) 150.121 6 (677.0-894.6) C
Red Blood Cell Count (#/mL)	5.415 (5.09-5.83) 0.261 6	6.210 (5.26-6.84) 0.628 5 (5.782-6.642) A	5.857 (5.30-6.29) 0.377 6 (5.464-6.250) A	5.852 (5.32-6.28) 0.377 5 (5.432-6.293) A	5.728 (5.06-6.04) 0.378 6 (5.335-6.121) A

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating significant differences (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 2. Descriptive Statistics* of Hematology Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
White Blood Cell Count (#/mL)	9.303 (6.38-12.40) 2.274 6	11.090 (8.35-12.50) 1.604 5 (8.708-14.180) A	9.355 (6.84-12.90) 2.353 6 (6.828-11.882) A	17.200 (12.2-22.4) 4.693 5 (14.317-19.789) B	17.083 (15.3-20.2) 1.975 6 (14.557-19.610) B

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating significant differences (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 3. Descriptive Statistics* of Thiodiglycol Parameters, by Study Day for the Six Animals Tested in Phase I, Part B.

Study Day	Urine Volume (mL)	Thiodiglycol Concentration (µg/mL)	Total Thiodiglycol (µg)
0 - AM	94.83 (17-220) 70.56 6 (14.98-174.68)	0.00 (0.00-0.00) 0.00 6 (-0.45-0.45) A	0.00 (0.00-0.00) 0.00 6 (-48.54-48.54) A
0- PM	175.17 (21-483) 177.47 6 (95.32-255.02)	2.14 (0.66-4.98) 1.66 6 (1.69-2.59) B	199.21 (72.82-362.94) 124.03 6 (150.67-247.75) B
1 - AM	36.17 (0-71) 24.15 6 (-43.68-116.02)	1.57 (0.09-3.16) 1.11 5 (1.08-2.05) B	61.92 (3.23-101.53) 37.01 5 (9.73-116.03) A
1 - PM	210.20 (67-369) 112.85 5 (125.97-297.47)	0.27 (0.05-1.05) 0.44 5 (-0.21-0.76) A	57.41 (7.40-233.10) 98.34 5 (5.43-111.73) A
2 - AM	103.75 (6.5-220) 85.80 6 (23.90-183.60)	0.65 (0.15-1.94) 0.71 6 (0.21-1.10) A	87.55 (6.46-426.80) 166.71 6 (39.01-136.10) A
2 - PM	194.40 (58-356) 144.72 5 (109.51-281.02)	0.21 (0.06-0.43) 0.16 5 (-0.27-0.70) A	26.20 (16.38-40.94) 9.54 5 (-27.59-78.71) A

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating statistical comparison to control (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 3. Descriptive Statistics* of Thiodiglycol Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Study Day	Urine Volume (mL)	Thiodiglycol Concentration (µg/mL)	Total Thiodiglycol (µg)
3 - AM	95.83 (14-190) 67.69 6 (15.98-175.68)	0.18 (0.07-0.38) 0.12 6 (-0.27-0.63) A	15.29 (3.50-35.91) 14.21 6 (-33.26-63.83) A
3 - PM	134.40 (46-337) 117.99 5 (49.51-221.02)	0.05 (0.00-0.11) 0.05 5 (-0.44-0.54) A	7.12 (0.00-18.54) 7.87 5 (-46.66-59.64) A
4 - AM	71.00 (20-115) 37.00 6 (-8.85-150.85)	0.04 (0.00-0.13) 0.05 6 (-0.40-0.49) A	3.55 (0.00-15.18) 5.81 6 (-44.99-52.09) A
4 - PM	189.67 (78-358) 99.80 6 (109.82-269.52)	0.03 (0.00-0.06) 0.02 6 (-0.42-0.47) A	6.24 (0.00-17.90) 6.94 6 (-42.30-54.78) A
5 - AM	78.83 (36-127) 30.80 6 (-1.02-158.68)	0.04 (0.00-0.12) 0.05 6 (-0.41-0.48) A	2.89 (0.00-9.68) 3.82 6 (-45.65-51.43) A
5 - PM	181.33 (72-371) 104.41 6 (101.48-261.18)	0.02 (0.00-0.12) 0.05 6 (-0.43-0.47) A	2.93 (0.00-17.55) 7.16 6 (-45.62-51.47) A

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating statistical comparison to control (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

Table 3. Descriptive Statistics* of Thiodiglycol Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Study Day	Urine Volume (mL)	Thiodiglycol Concentration (µg/mL)	Total Thiodiglycol (µg)
6 - AM	114.33 (64-198) 49.56 6 (34.48-194.18)	0.02 (0.00-0.08) 0.03 6 (-0.43-0.47) A	2.48 (0.00-8.01) 3.86 6 (-46.06-51.02) A
6 - PM	201.33 (85-453) 143.41 6 (121.48-281.18)	0.01 (0.00-0.05) 0.02 6 (-0.44-0.46) A	2.16 (0.00-12.96) 5.29 6 (-46.38-50.70) A
7 - AM	185.83 (72-318) 91.06 6 (105.98-265.68)	0.03 (0.00-0.08) 0.04 6 (-0.42-0.48) A	4.74 (0.00-12.21) 5.42 6 (-43.80-53.28) A
7 - PM	247.50 (165-339) 87.86 4 (146.29-334.07)	0.01 (0.00-0.03) 0.02 4 (-0.54-0.55) A	2.80 (0.00-11.19) 5.59 4 (-56.09-62.70) A

* MEAN
(MIN-MAX)
STD
N
(95% CI)
Letter indicating statistical comparison to control (means for study days sharing at least one common letter were not significantly different at the 0.05 level for the given parameter)

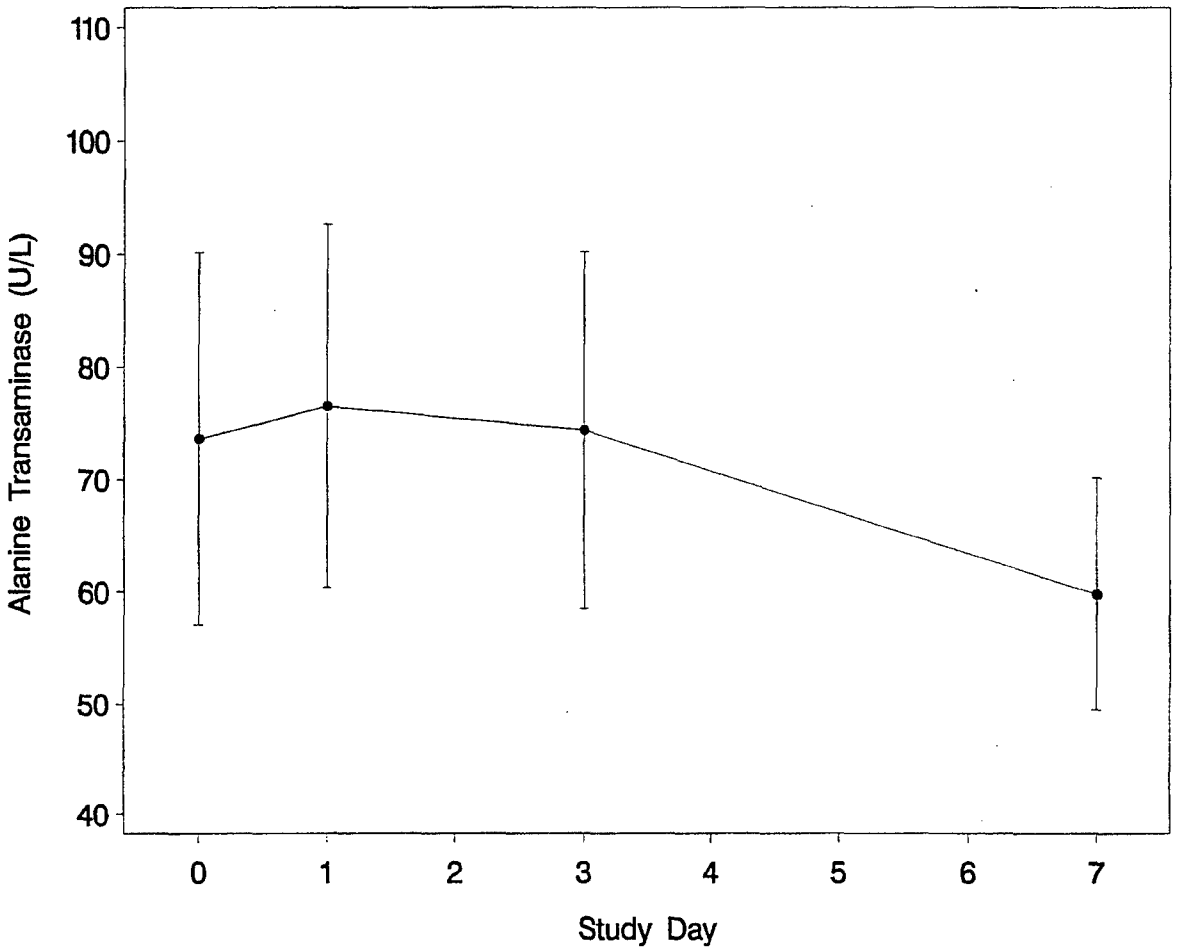

Figure A-1. Mean ± 2 Standard Errors Alanine Transaminase (U/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

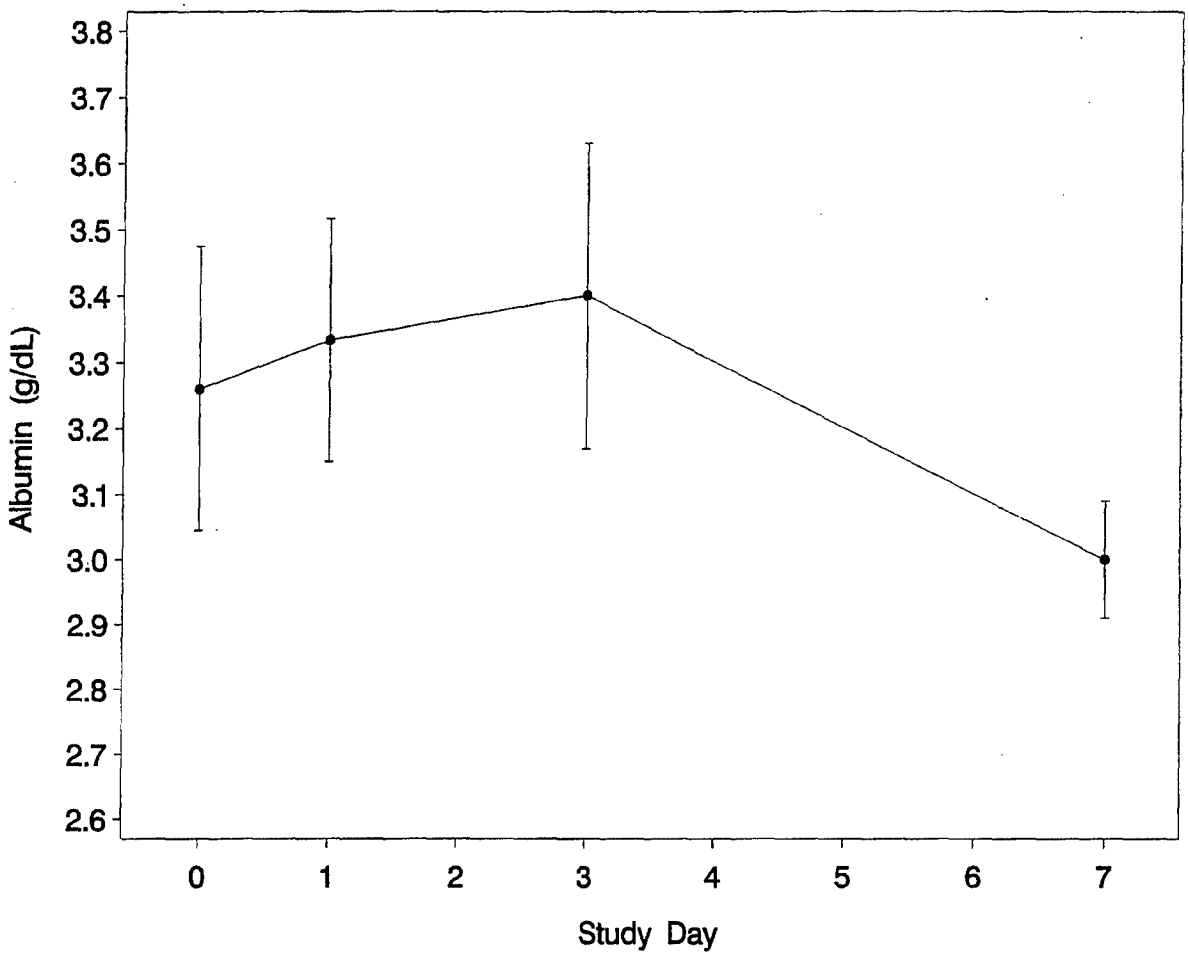

Figure A-2. Mean ± 2 Standard Errors Albumin (g/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

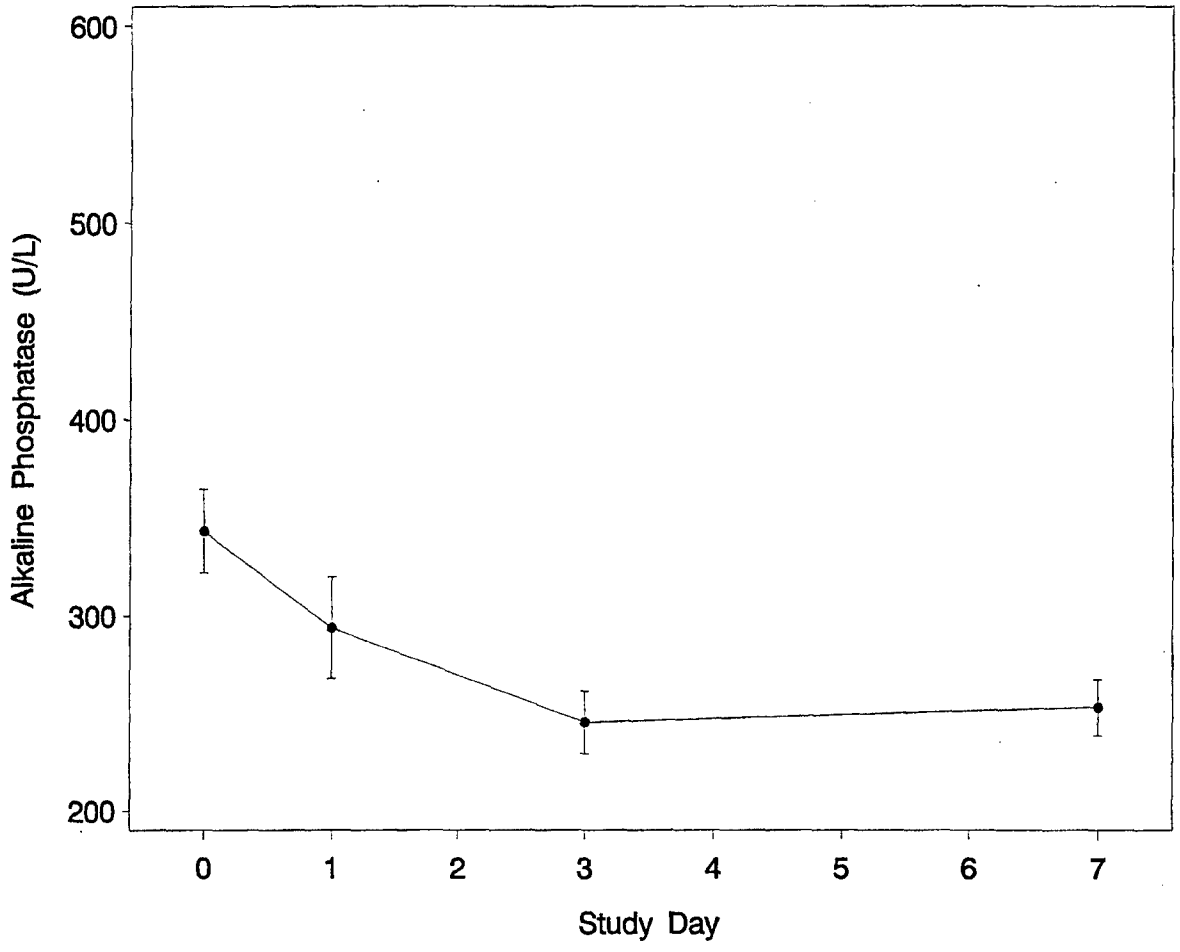

Figure A-3. Mean ± 2 Standard Errors Alkaline Phosphatase (U/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

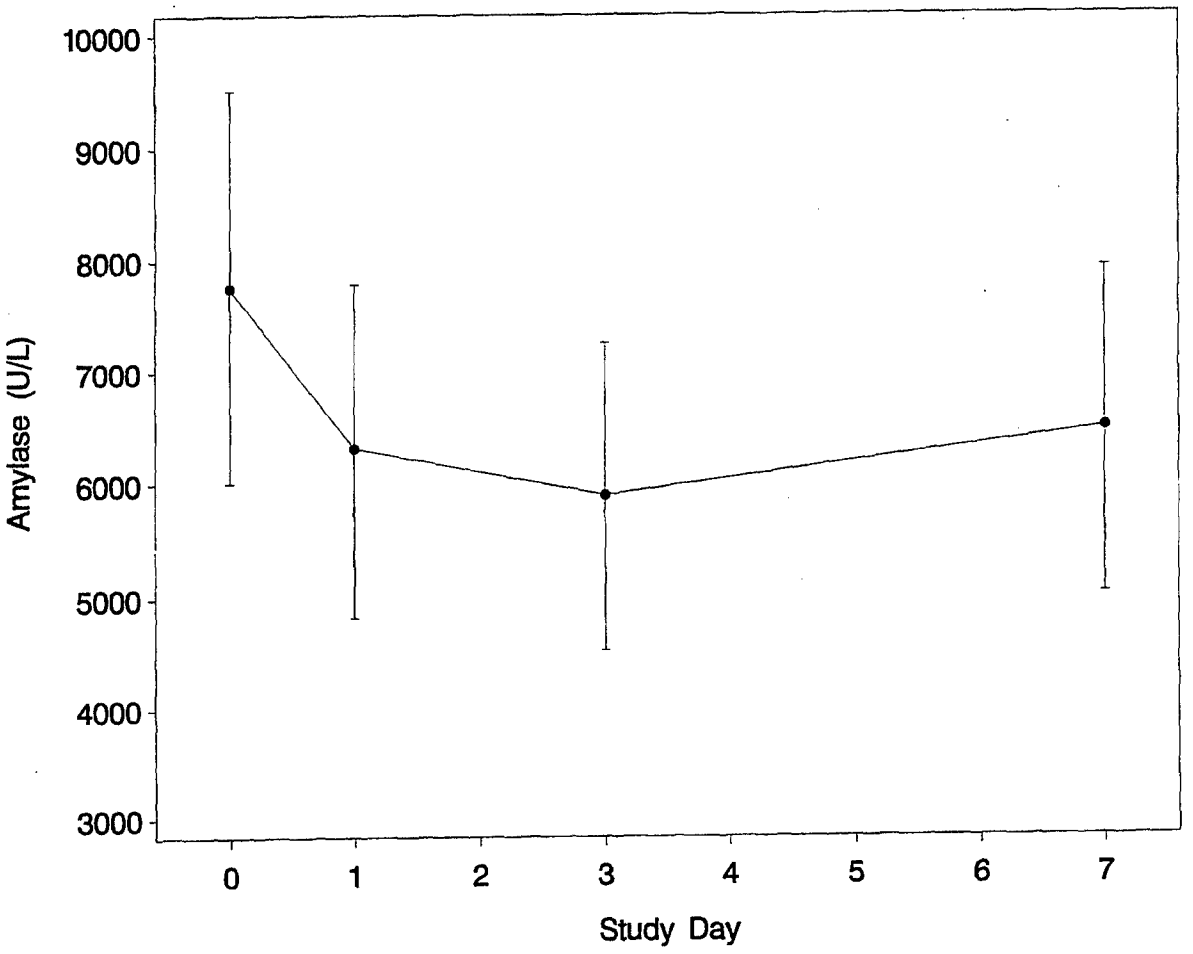

Figure A-4. Mean ± 2 Standard Errors Amylase (U/L) by Study Day for the Six Animals Tested in Phase 1, Part B

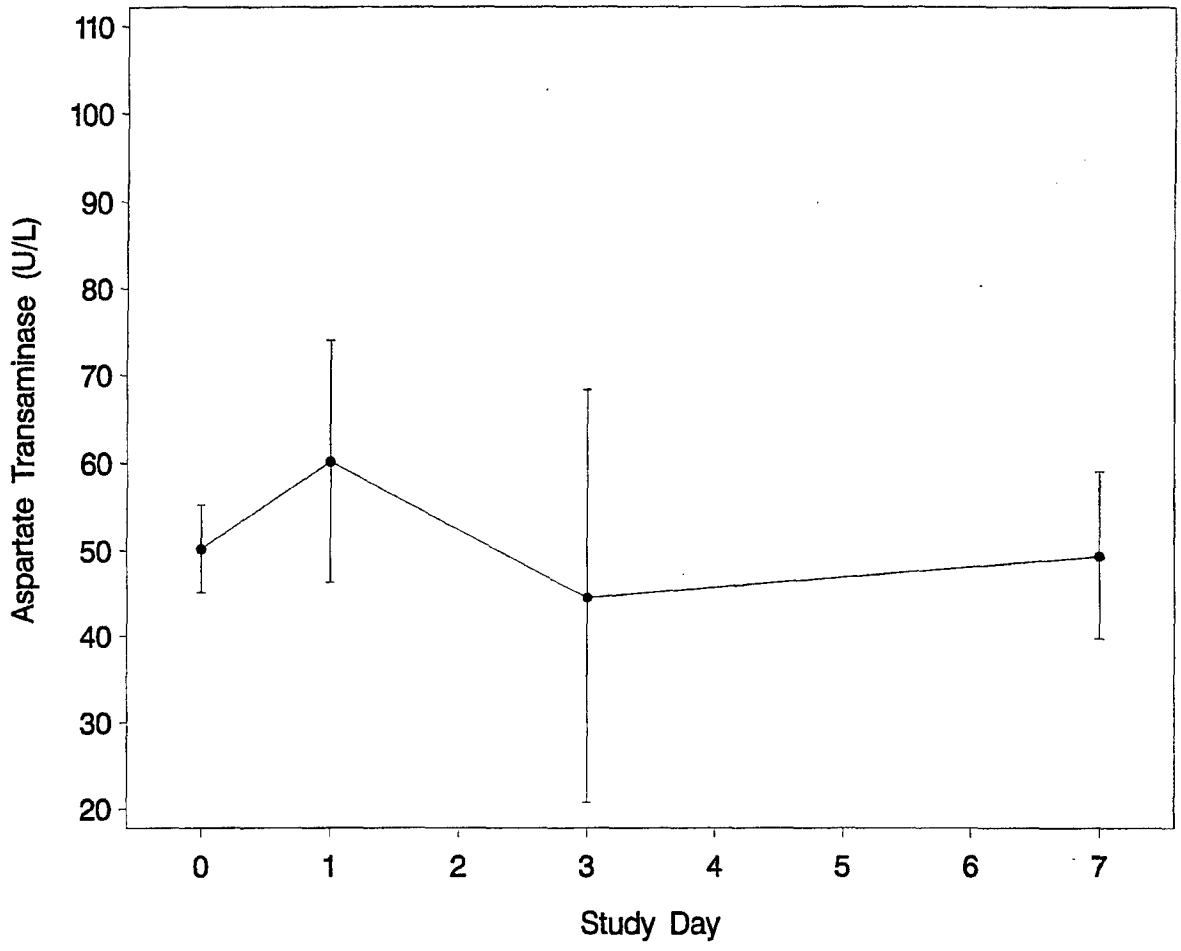

Figure A-5. Mean ± 2 Standard Errors Aspartate Transaminase (U/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

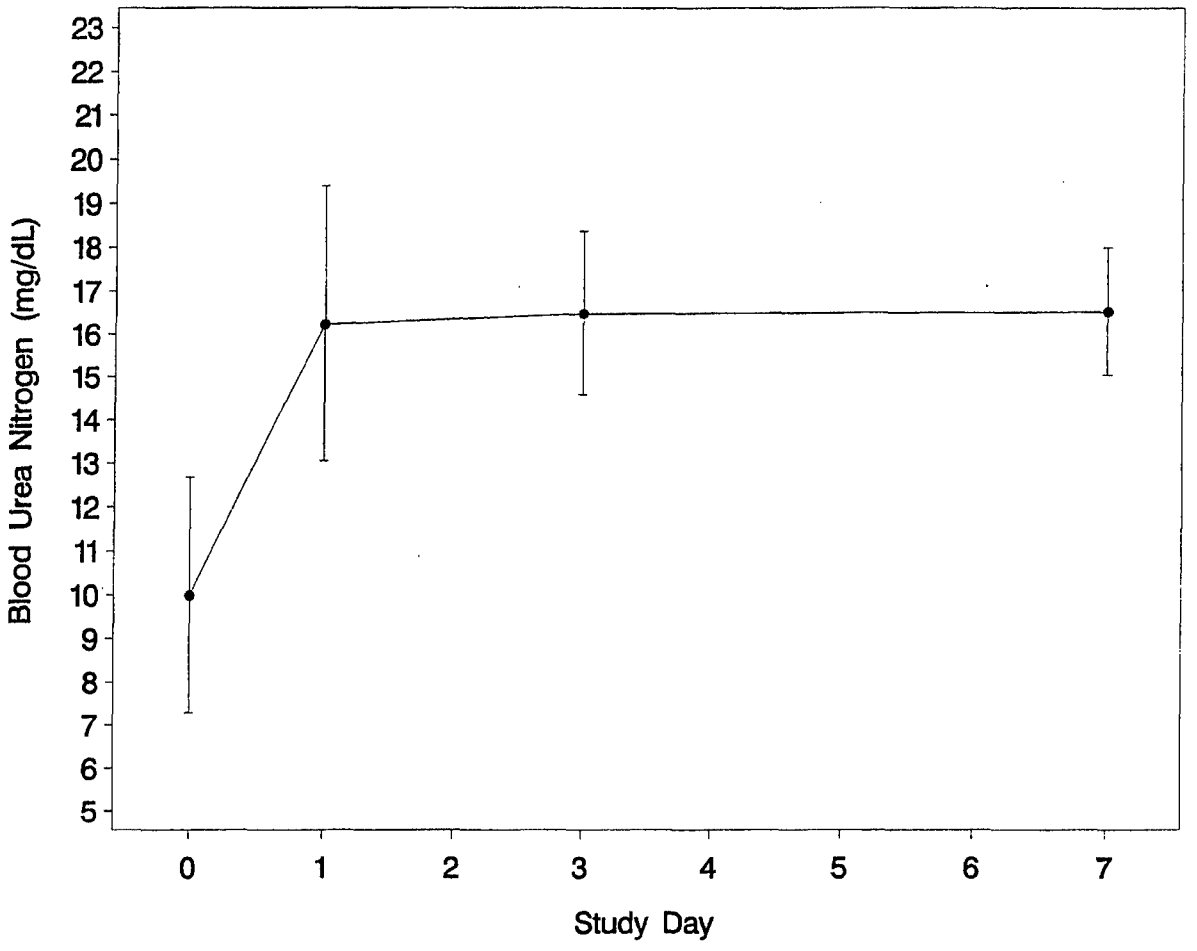

Figure A-6. Mean ± 2 Standard Errors Blood Urea Nitrogen (mg/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

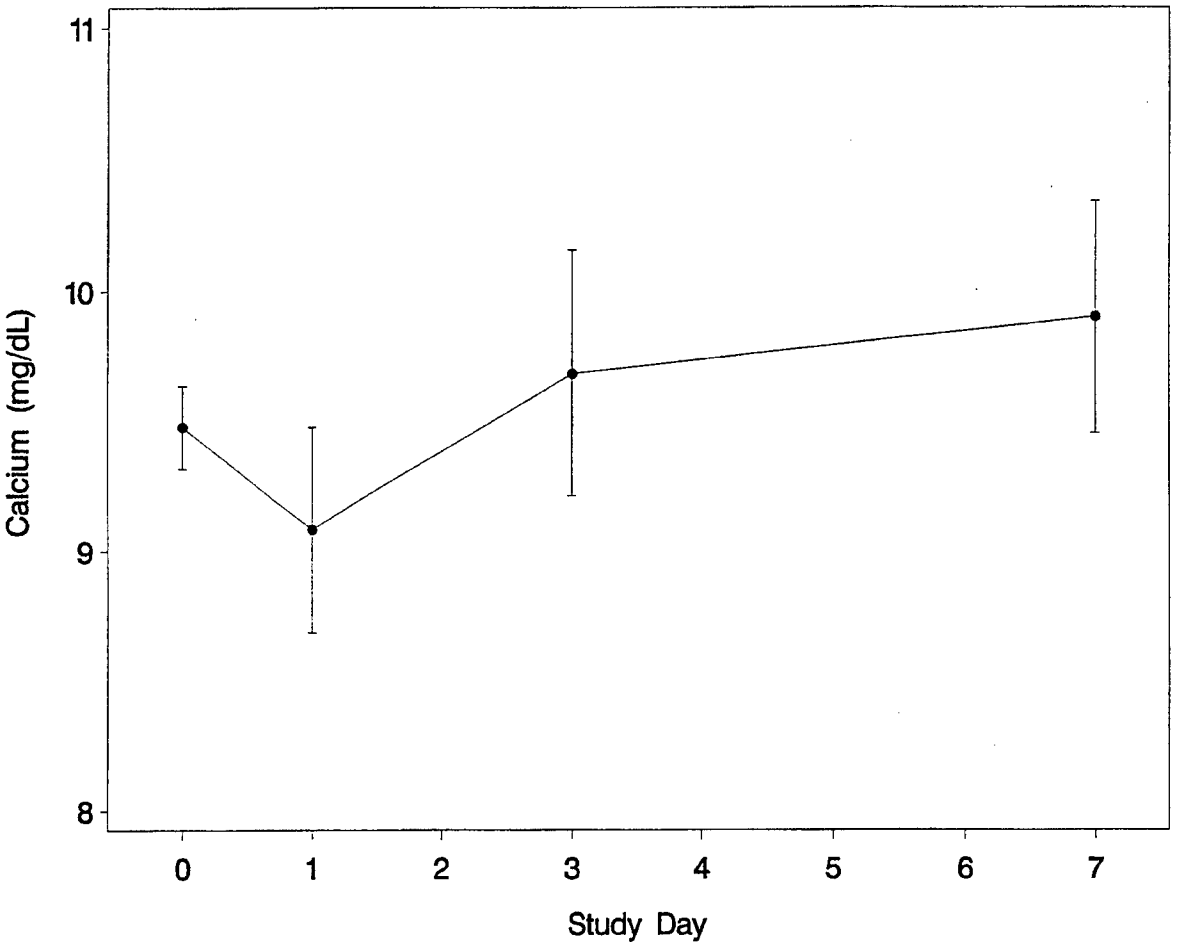

Figure A-7. Mean ± 2 Standard Errors Calcium (mg/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

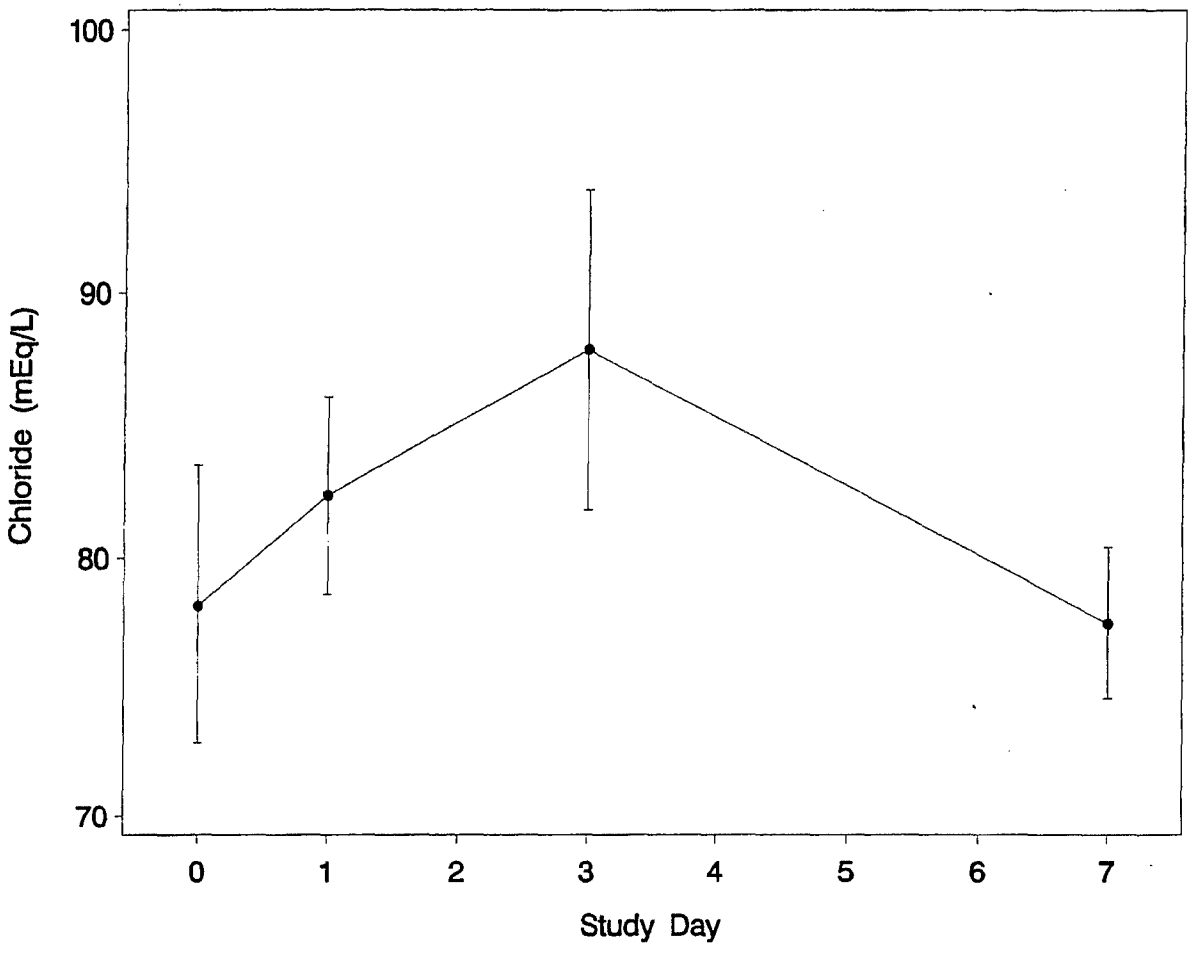

Figure A-8. Mean ± 2 Standard Errors Chloride (mEq/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

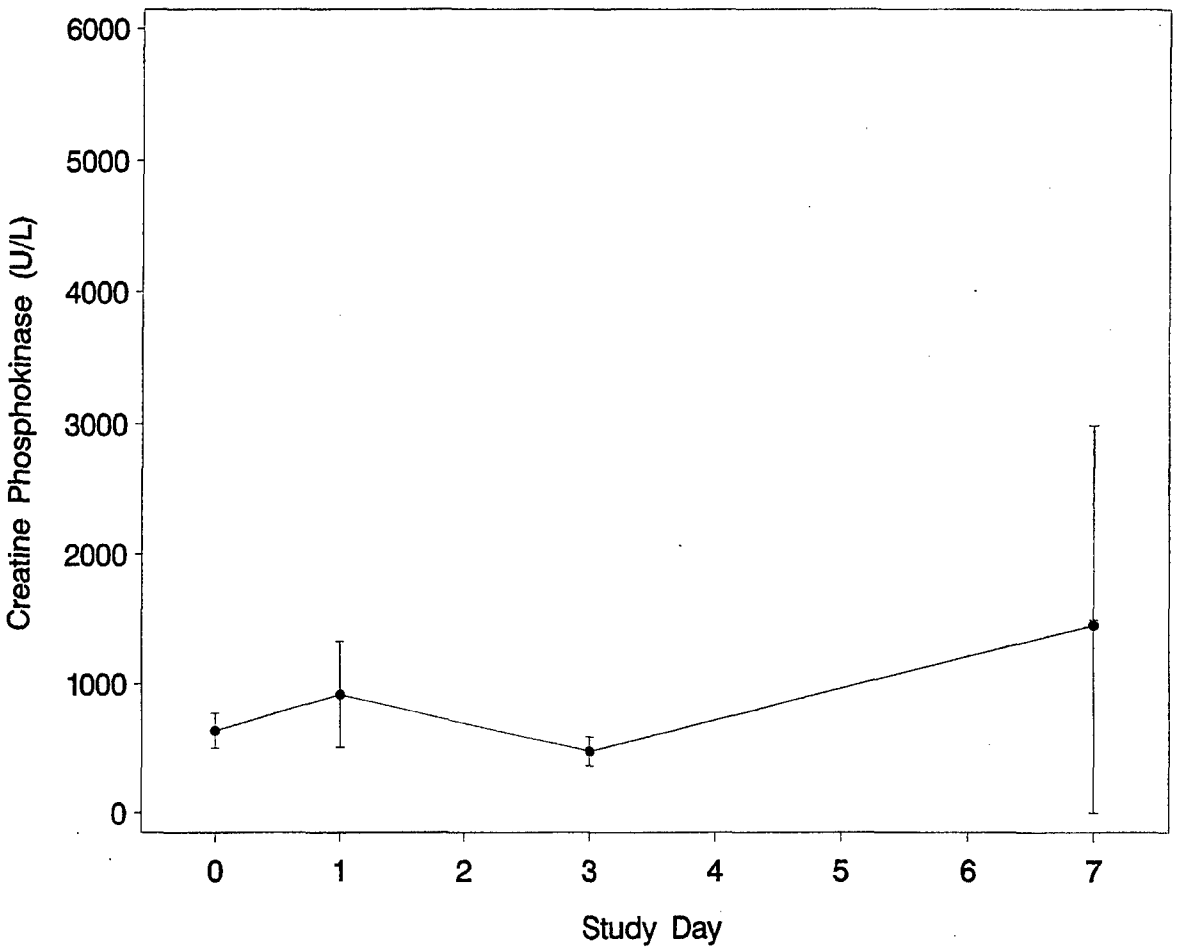

Figure A-9. Mean ± 2 Standard Errors Creatine Phosphokinase (U/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

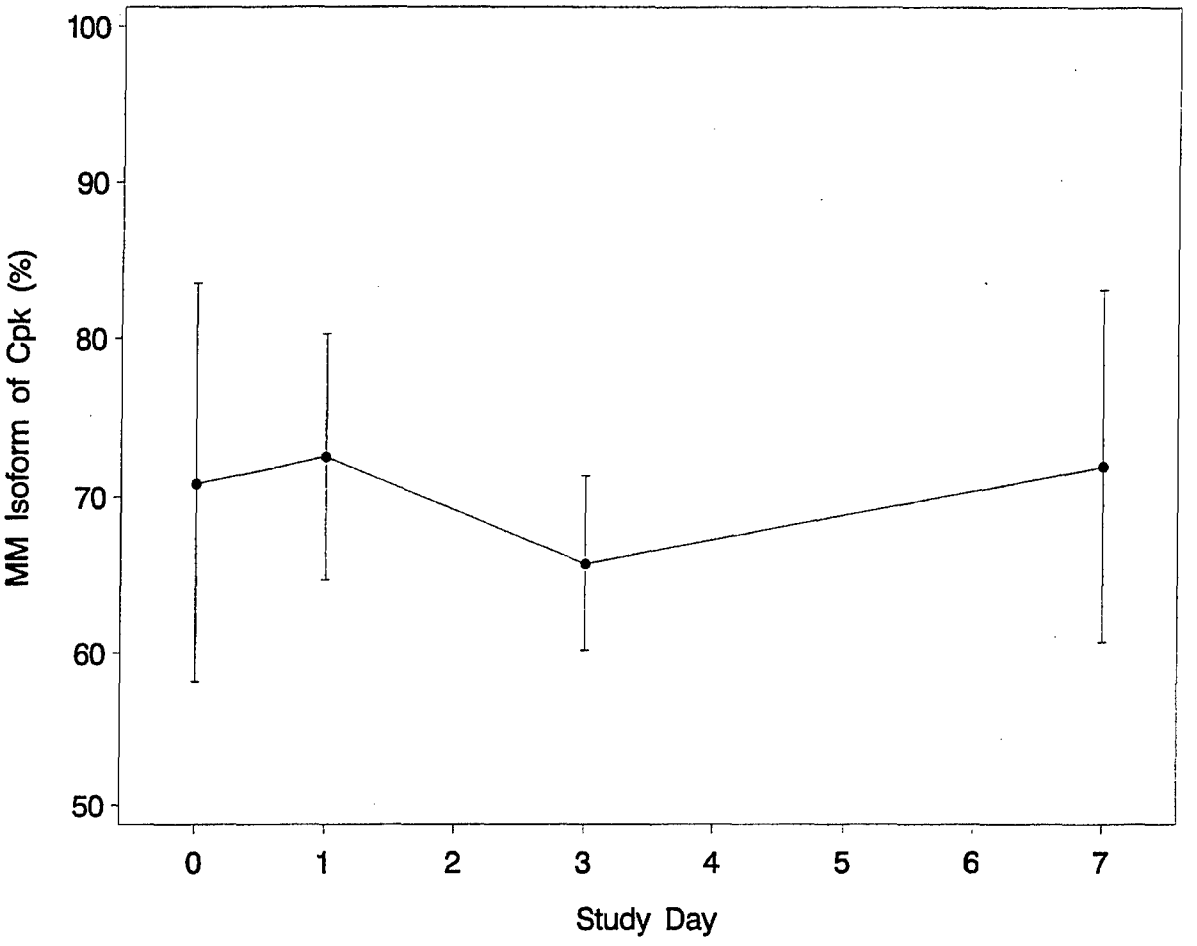

Figure A-10. Mean ± 2 Standard Errors MM Isoform of Creatine Phosphokinase (%) by Study Day for the Six Animals Tested in Phase 1, Part B.

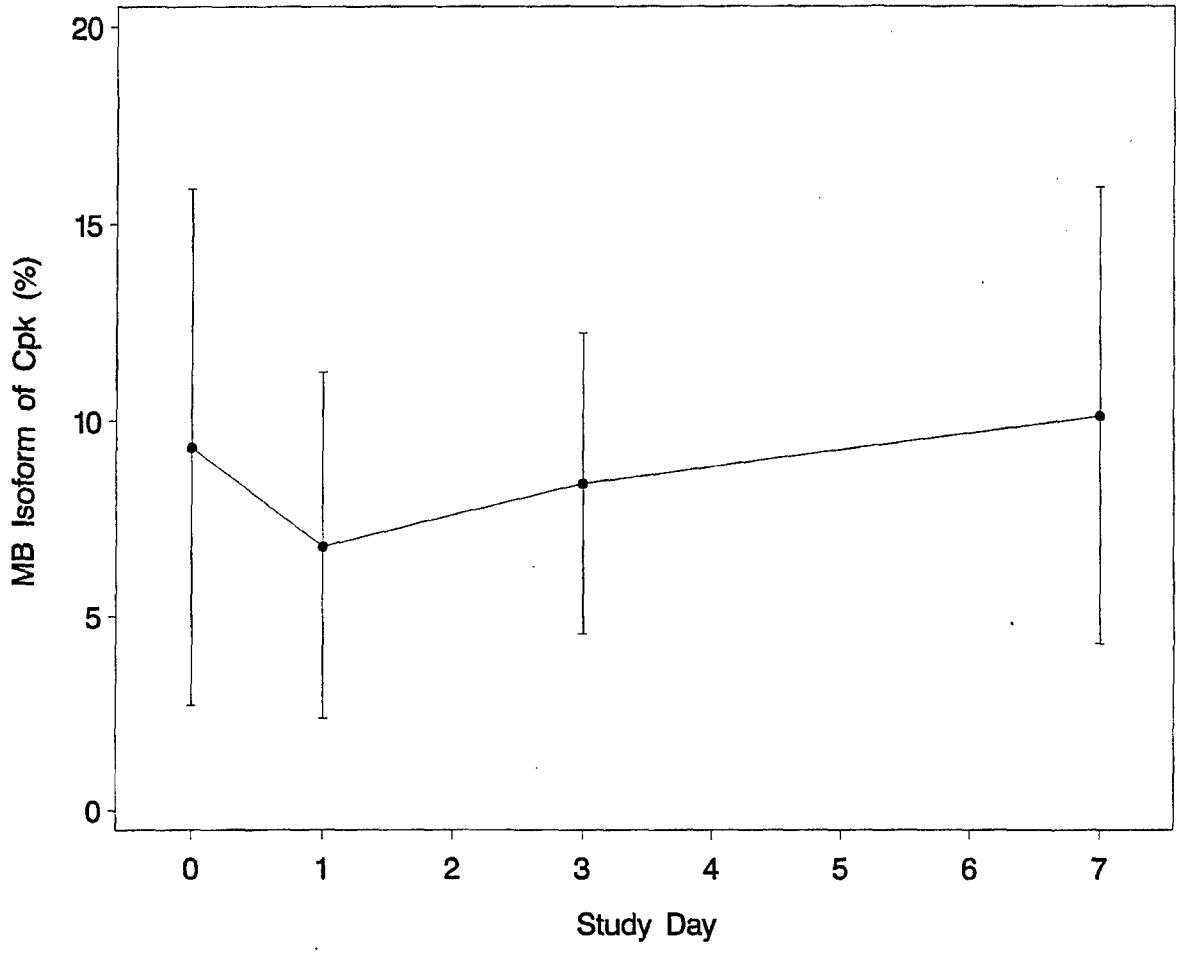

Figure A-11. Mean ± 2 Standard Errors MB Isoform of Creatine Phosphokinase (%) by Study Day for the Six Animals Tested in Phase 1, Part B.

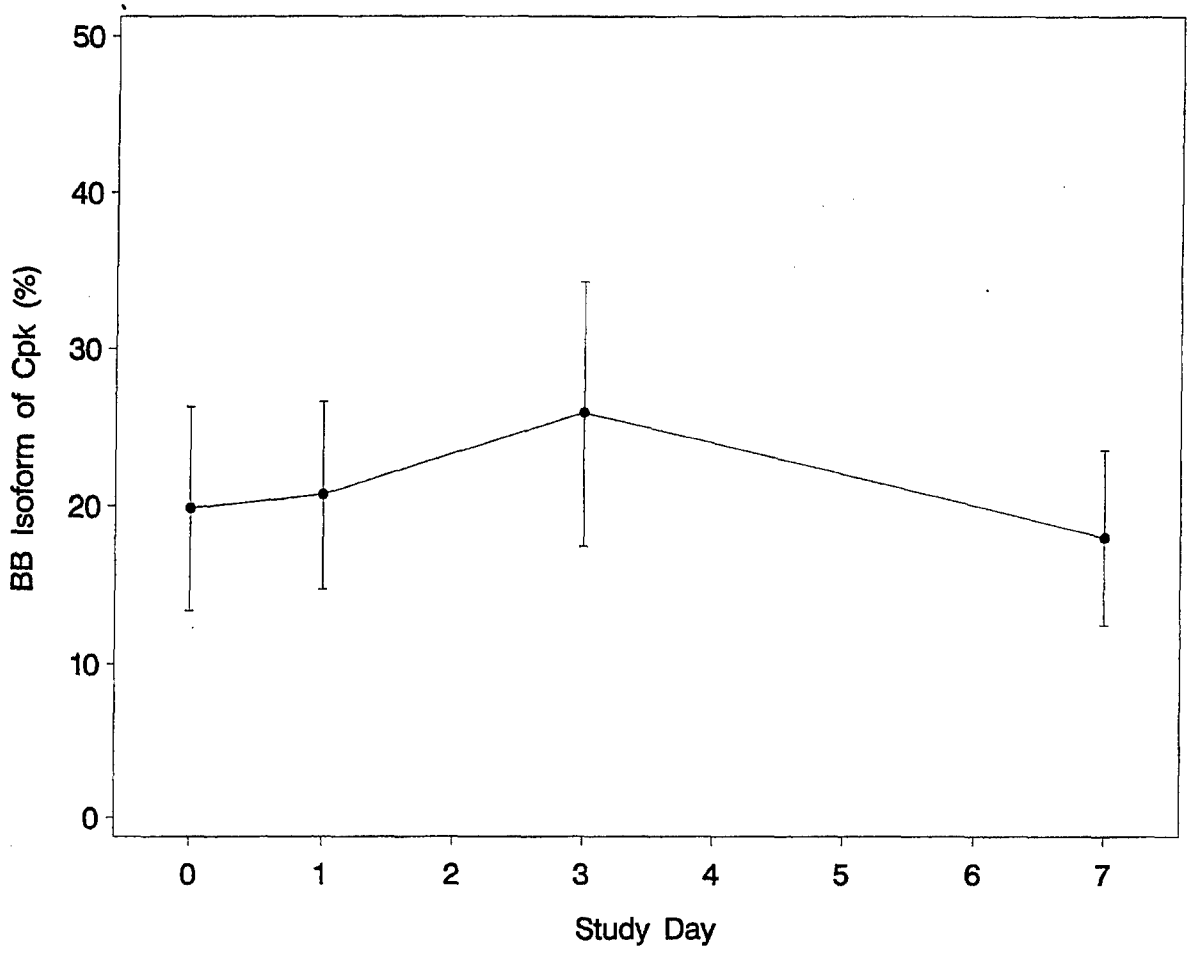

Figure A-12. Mean ± 2 Standard Errors BB Isoform of Creatine Phosphokinase (%) by Study Day for the Six Animals Tested in Phase 1, Part B.

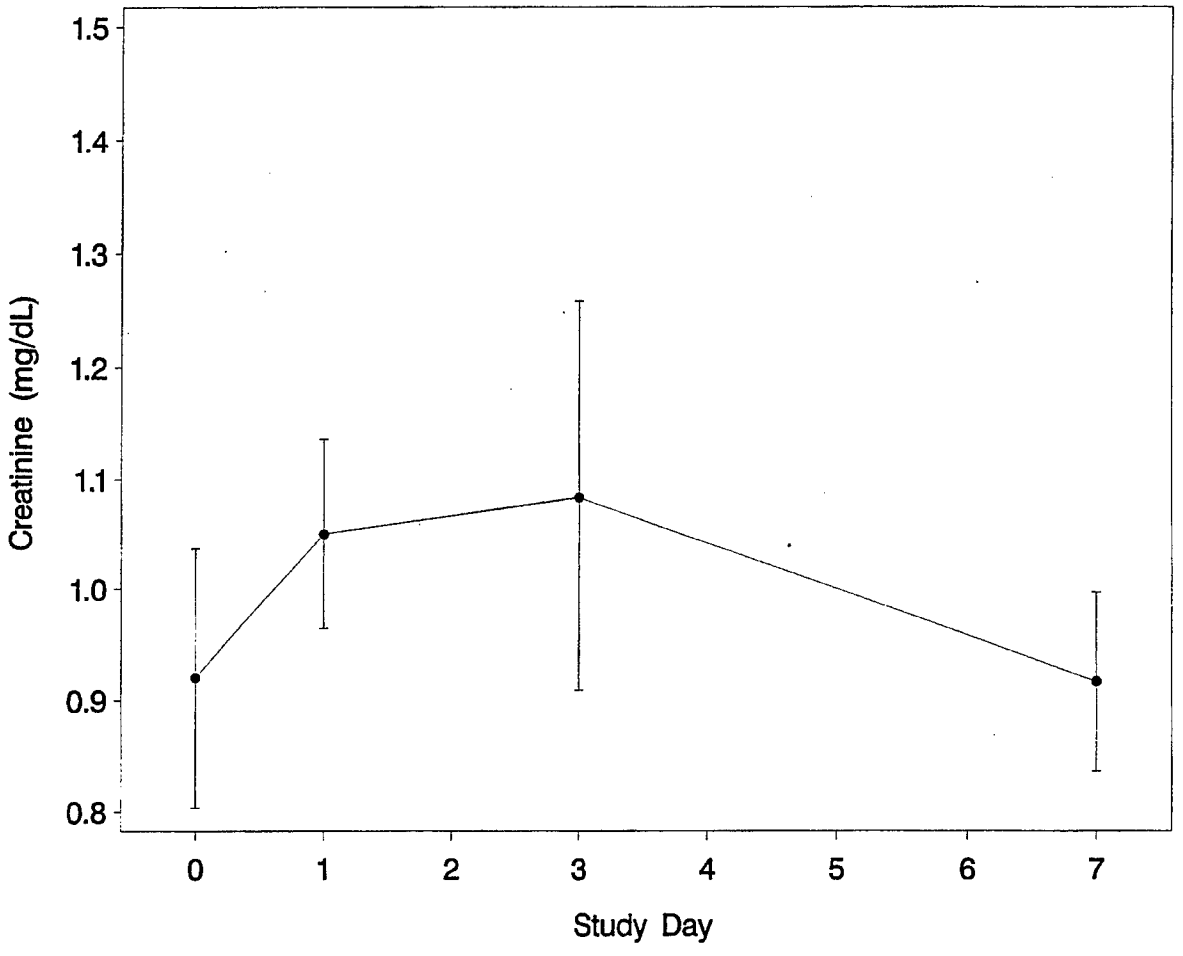

Figure A-13. Mean ± 2 Standard Errors Creatinine (mg/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

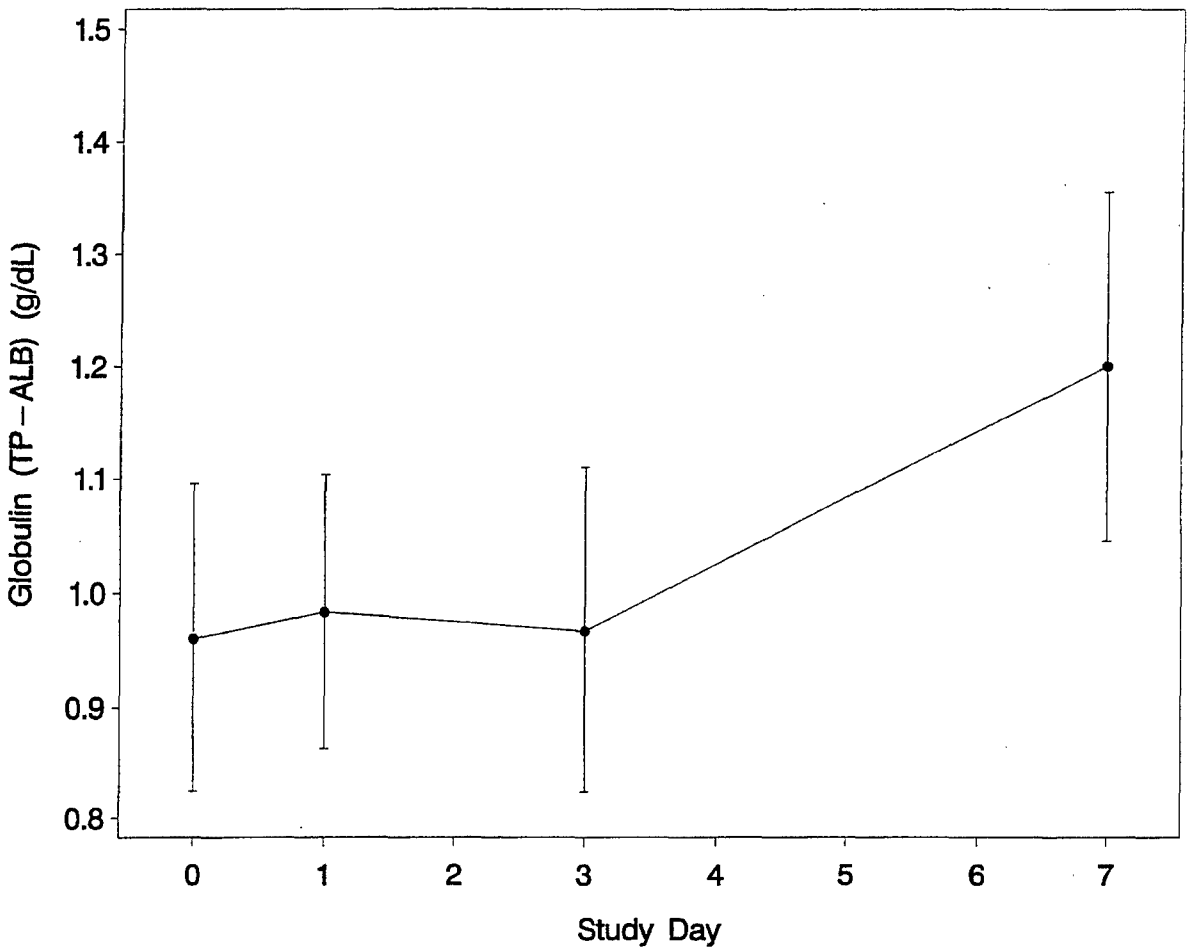

Figure A-14. Mean ± 2 Standard Errors Globulin (TP-ALB) (g/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

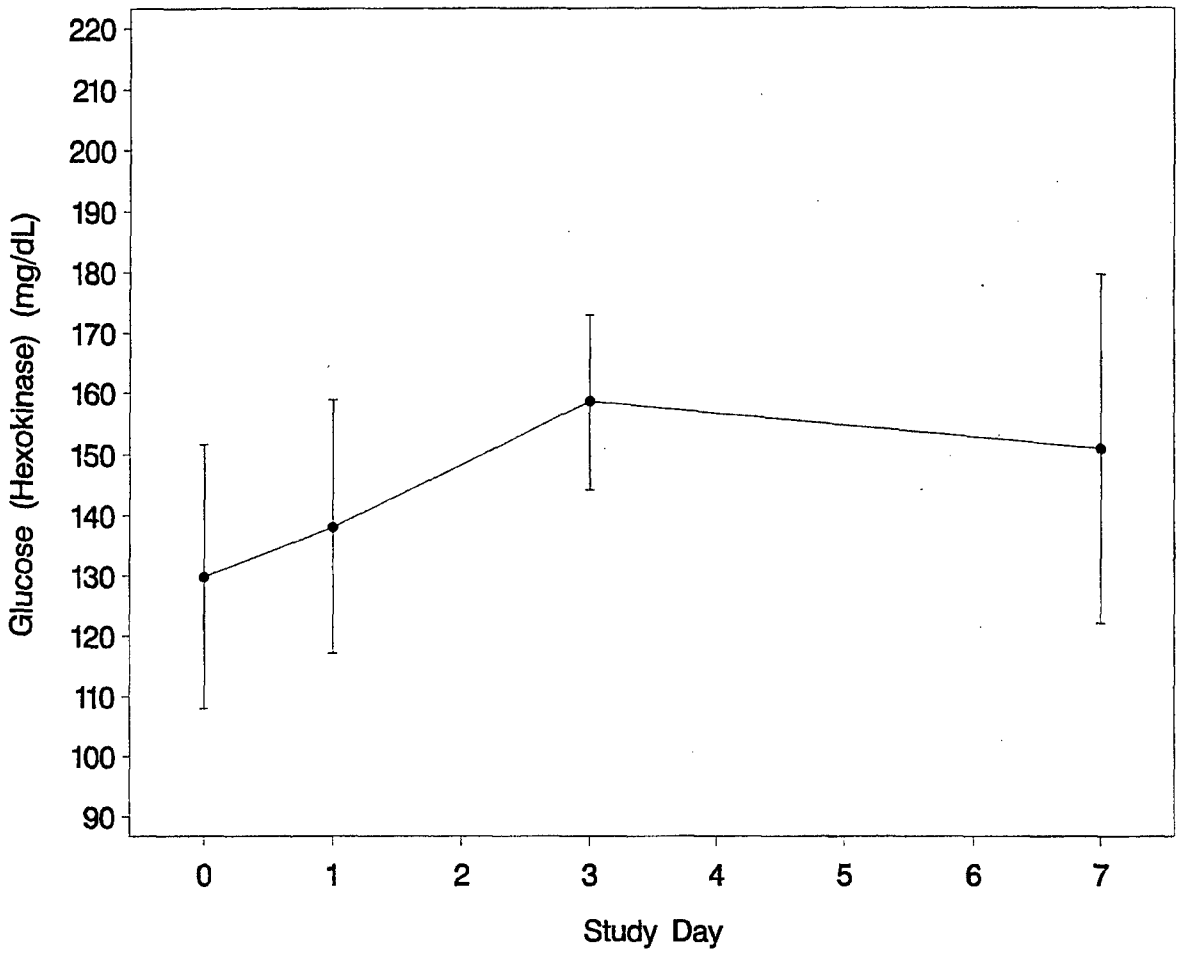

Figure A-15. Mean ± 2 Standard Errors Glucose (Hexokinase) (mg/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

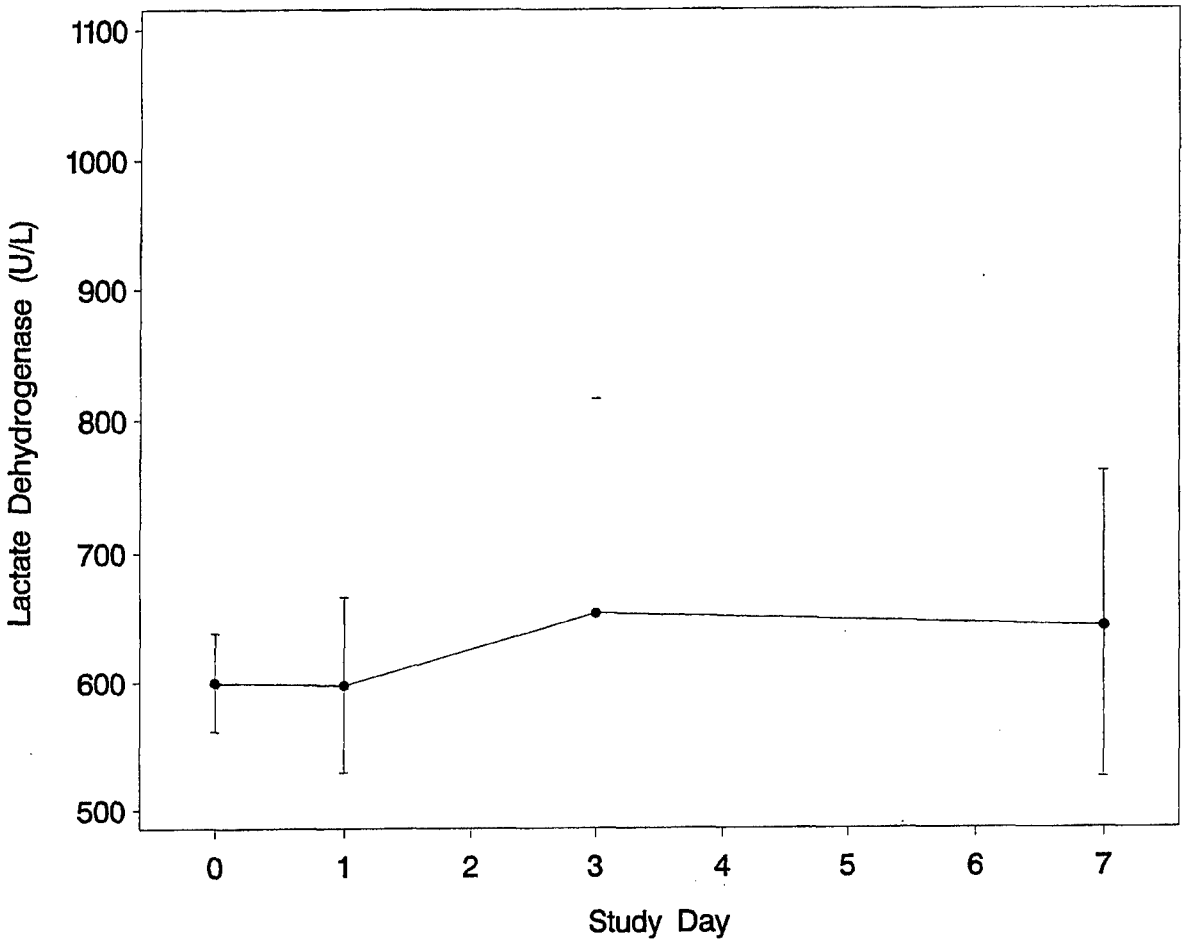

Figure A-16. Mean ± 2 Standard Errors Lactate Dehydrogenase (U/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

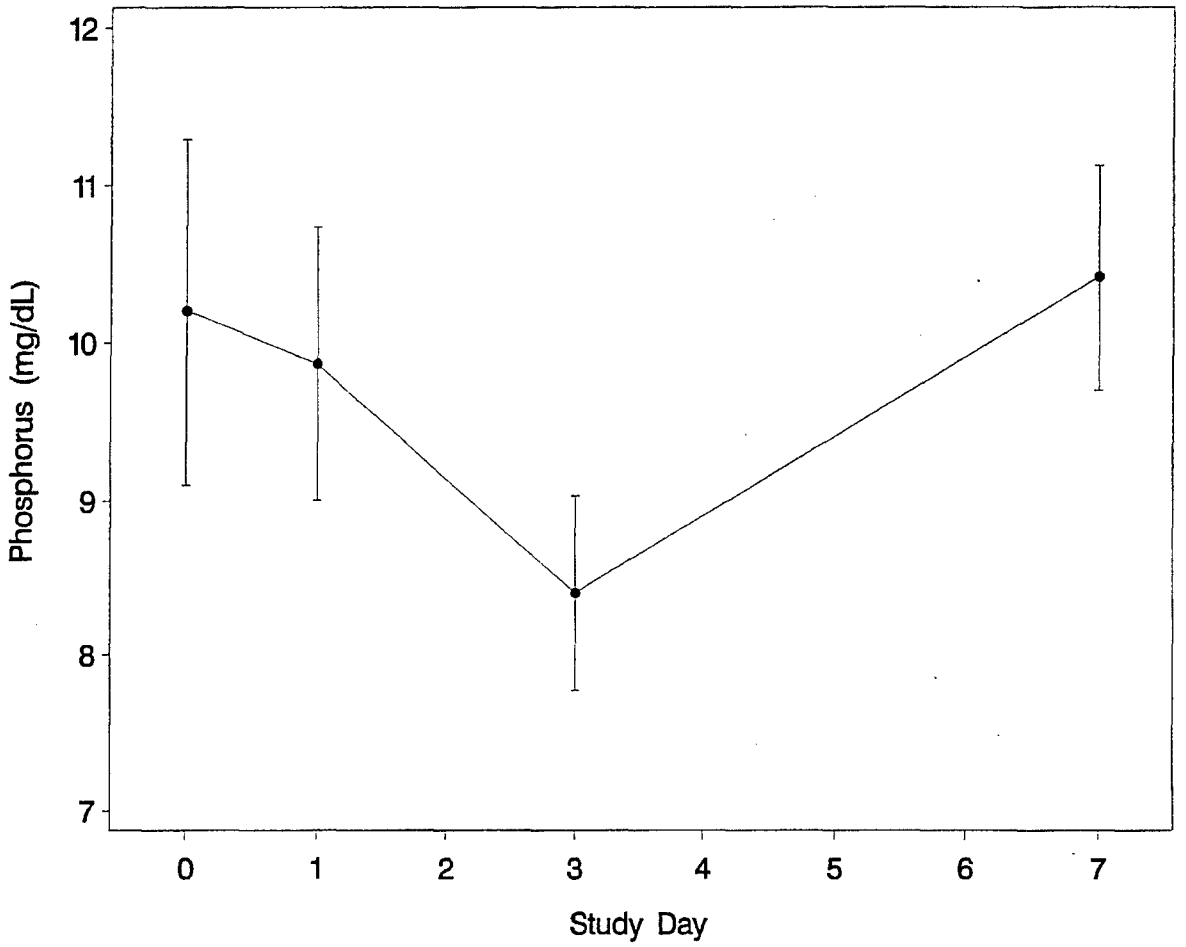

Figure A-17. Mean ± 2 Standard Errors Phosphorus (mg/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

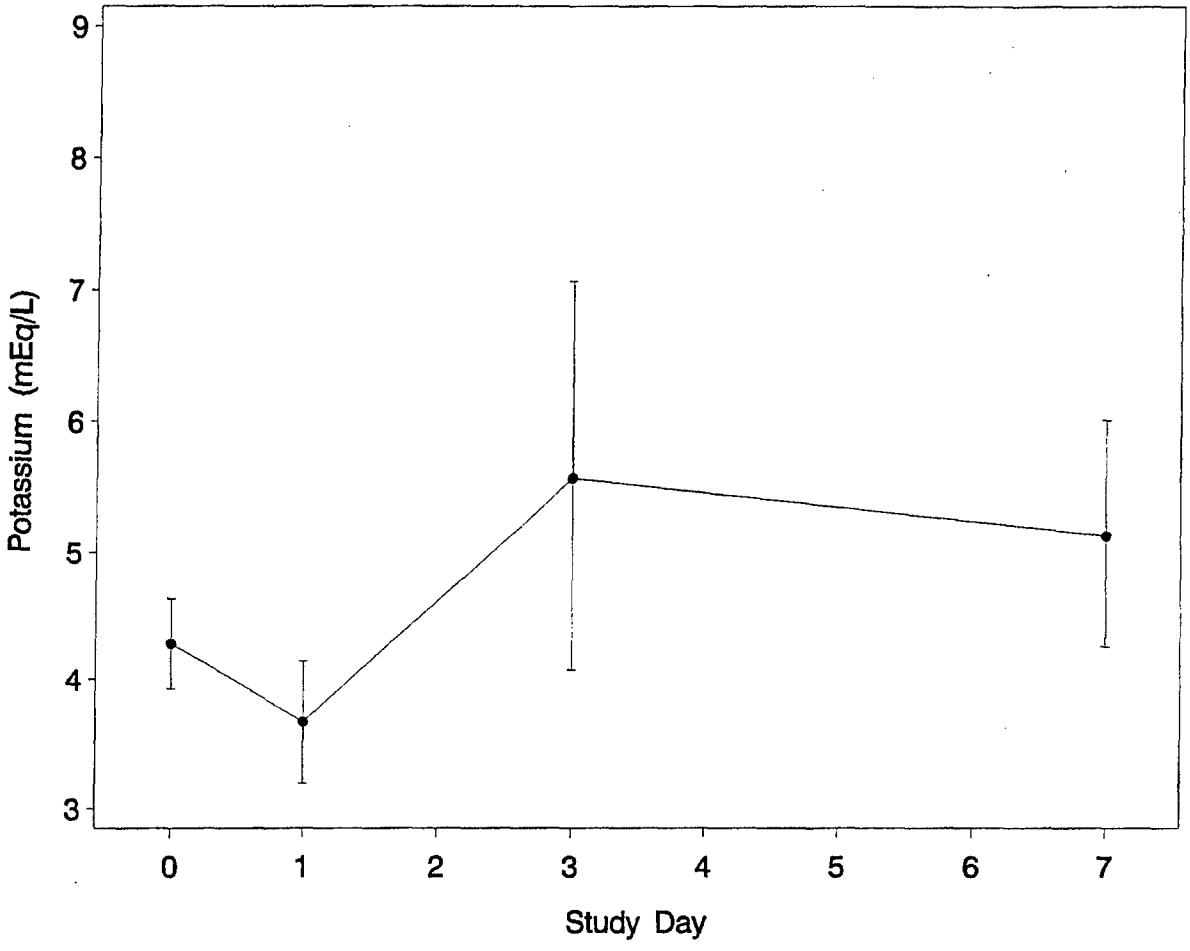

Figure A-18. Mean ± 2 Standard Errors Potassium (mEq/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

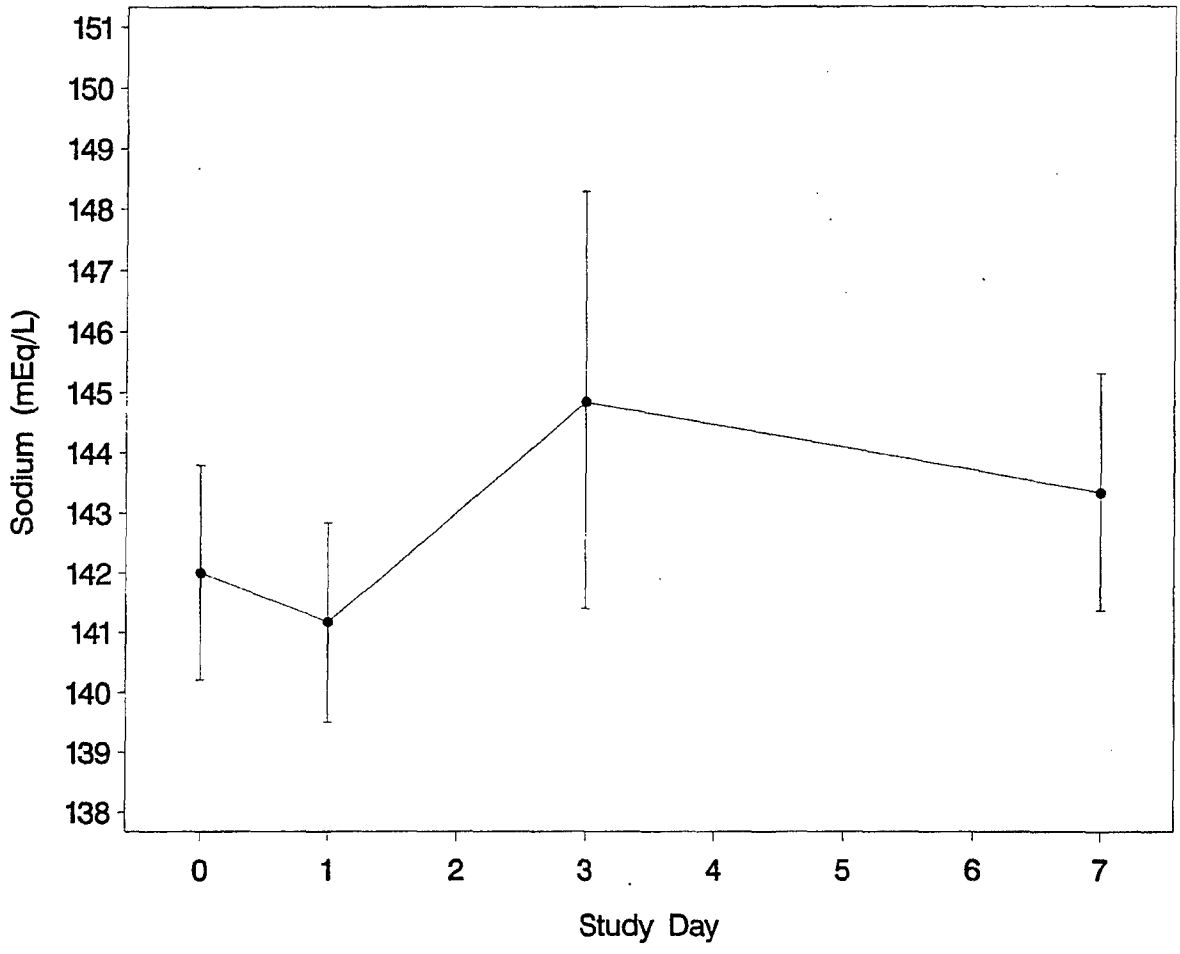

Figure A-19. Mean ± 2 Standard Errors Sodium (mEq/L) by Study Day for the Six Animals Tested in Phase 1, Part B.

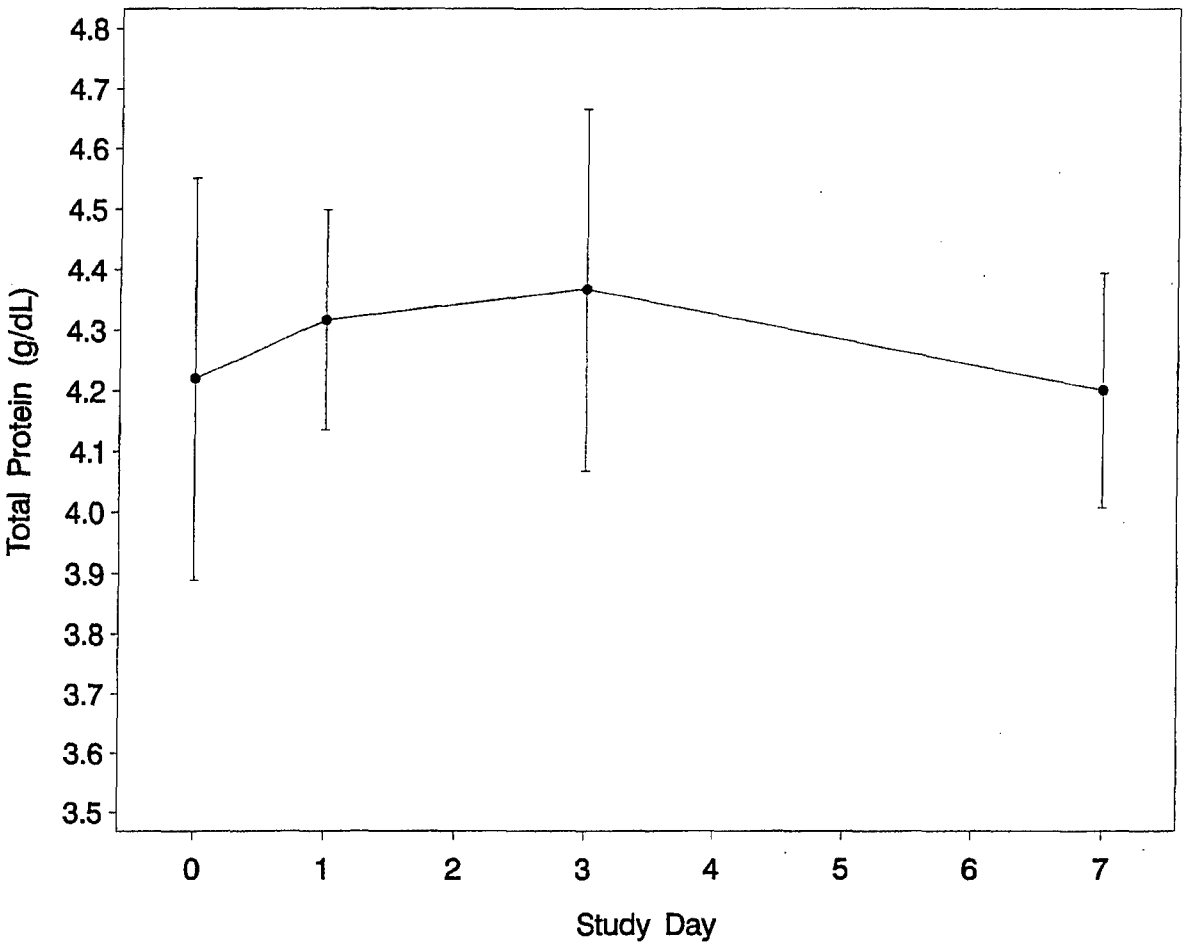

Figure A-20. Mean ± 2 Standard Errors Total Protein (g/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

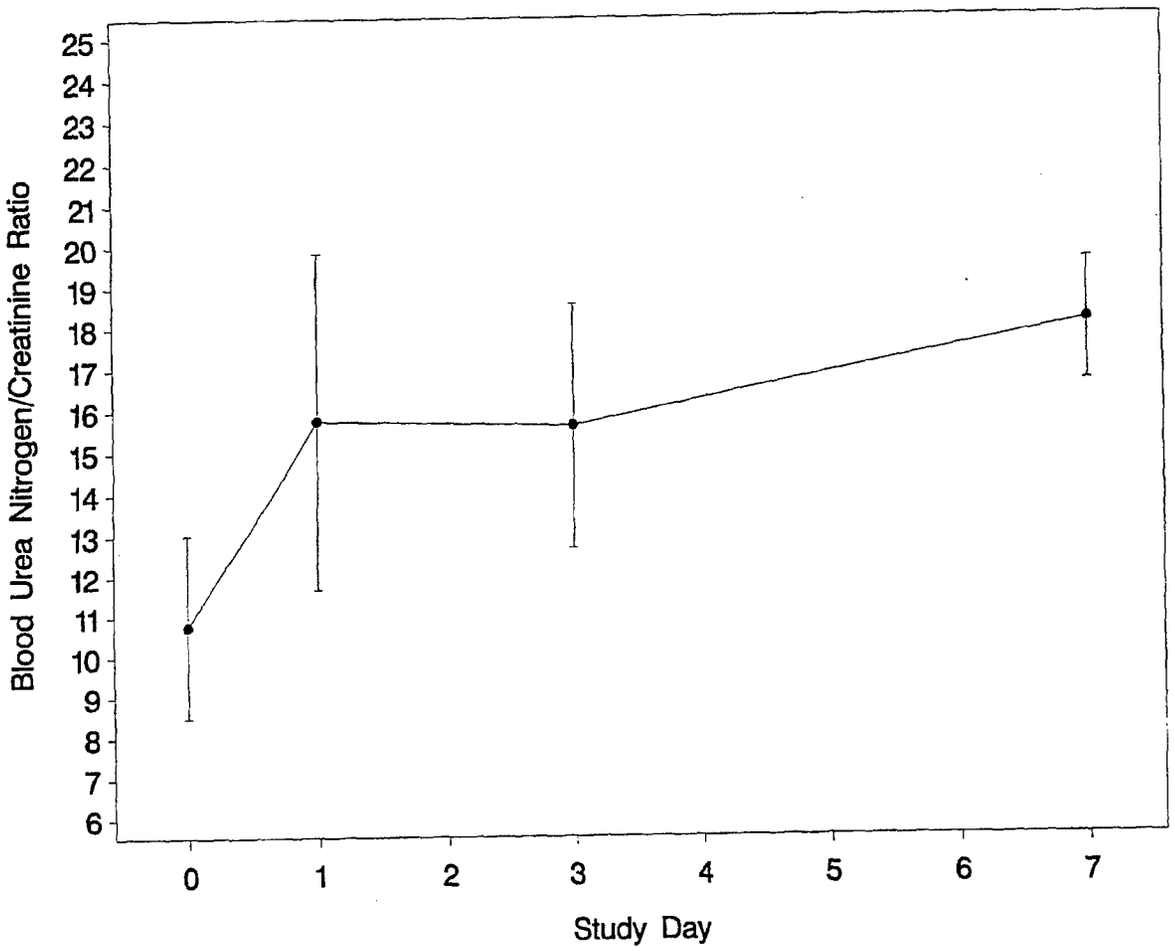

Figure A-21. Mean ± 2 Standard Errors Ratio of Blood Urea Nitrogen to Creatinine by Study Day for the Six Animals Tested in Phase 1, Part B.

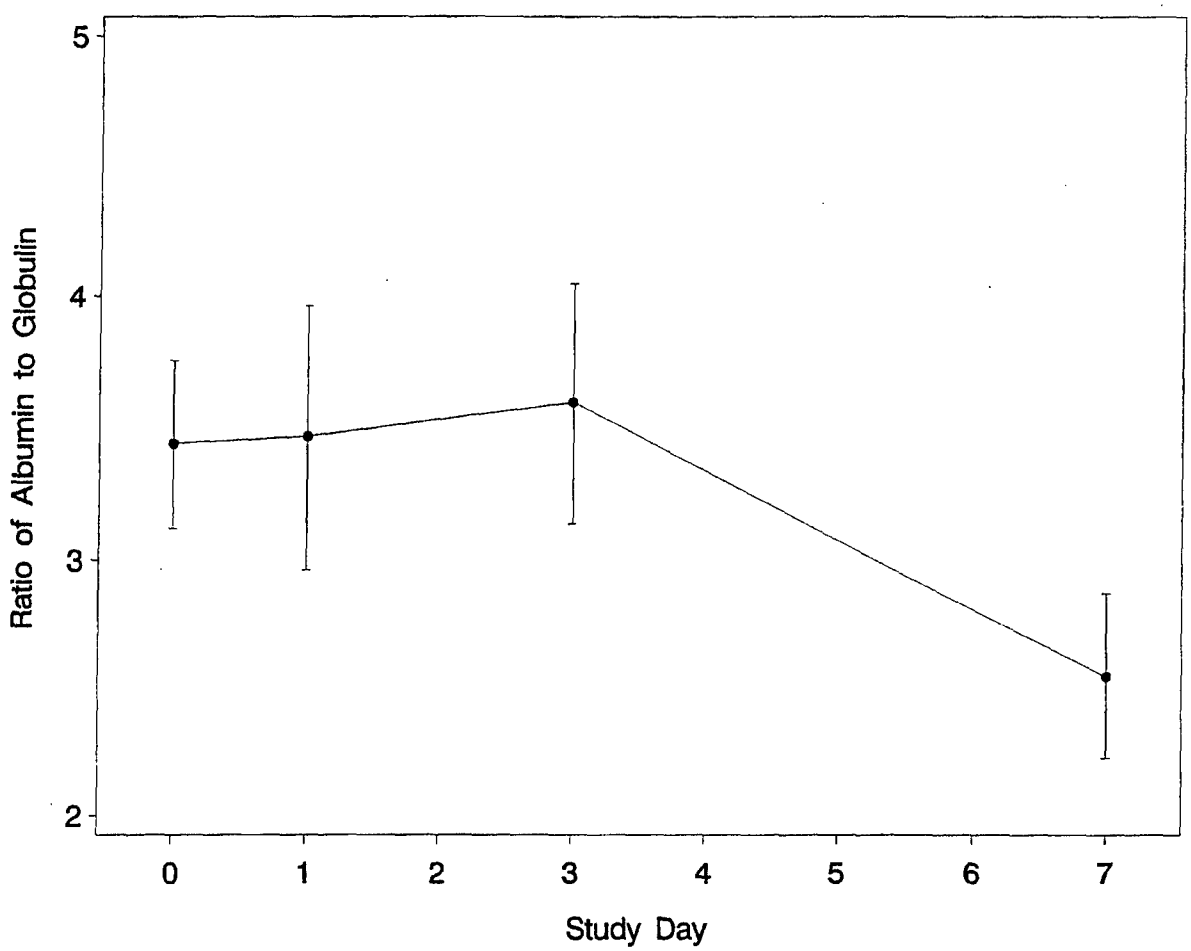

Figure A-22. Mean ± 2 Standard Errors Albumin to Globulin Ratio by Study Day for the Six Animals Tested in Phase 1, Part B.

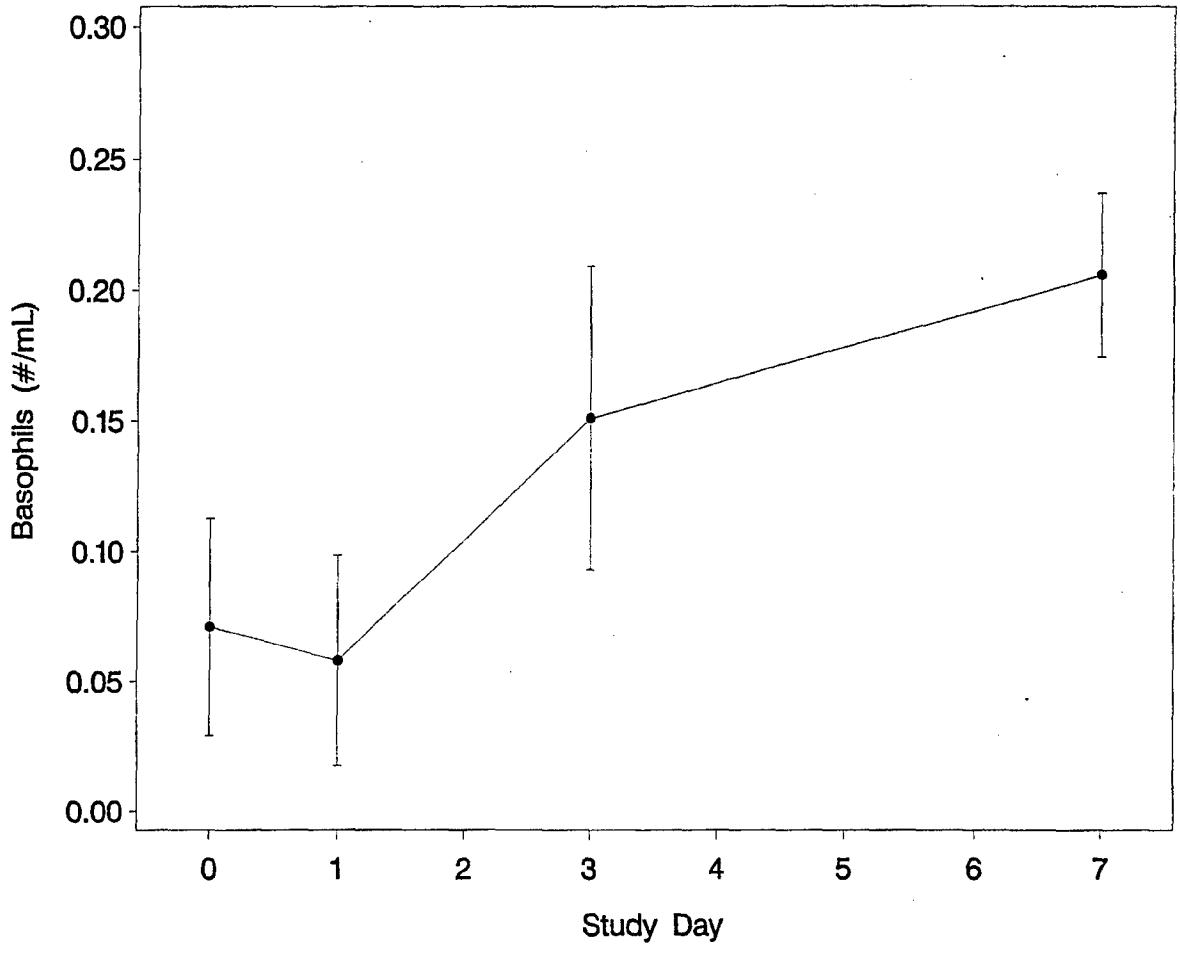

Figure A-23. Mean ± 2 Standard Errors Basophils (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

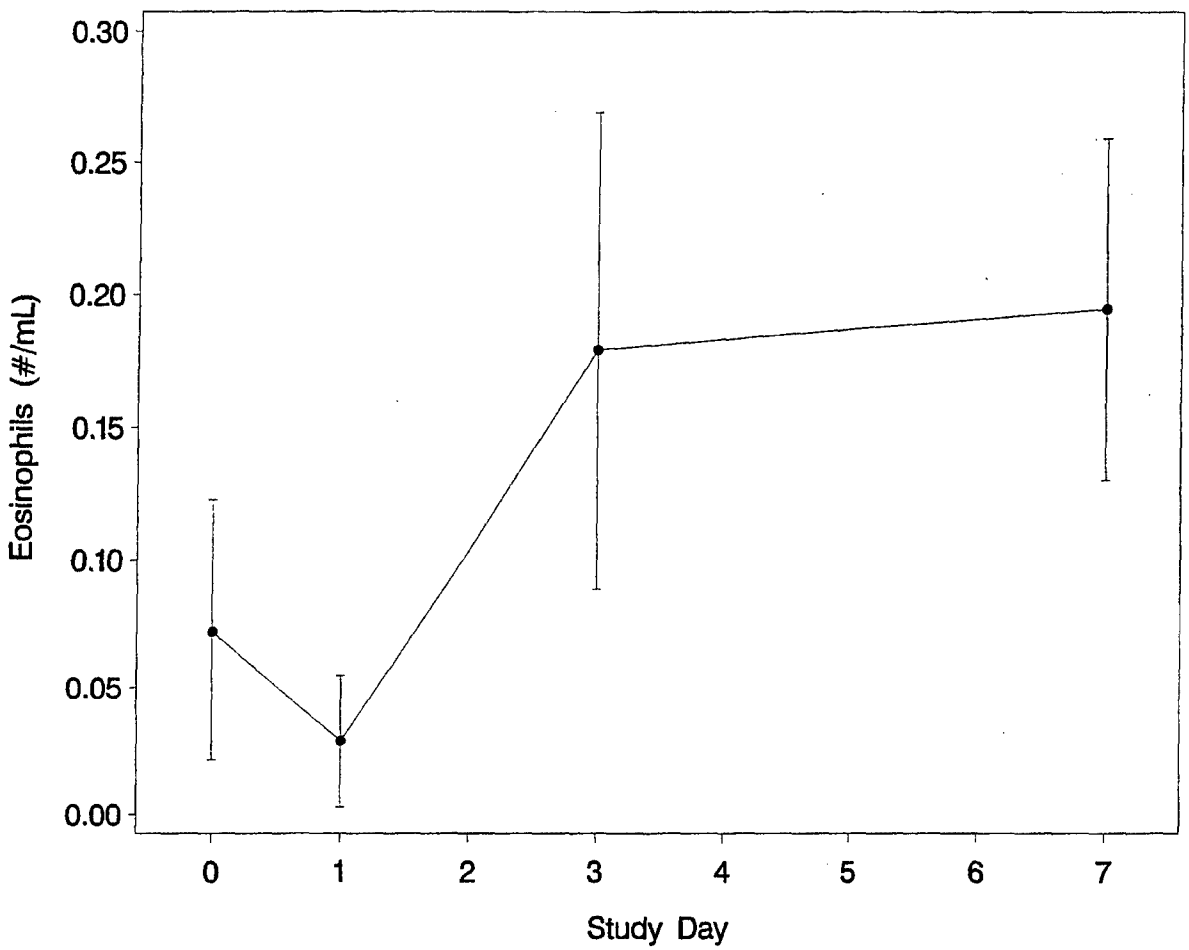

Figure A-24. Mean ± 2 Standard Errors Eosinophils (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

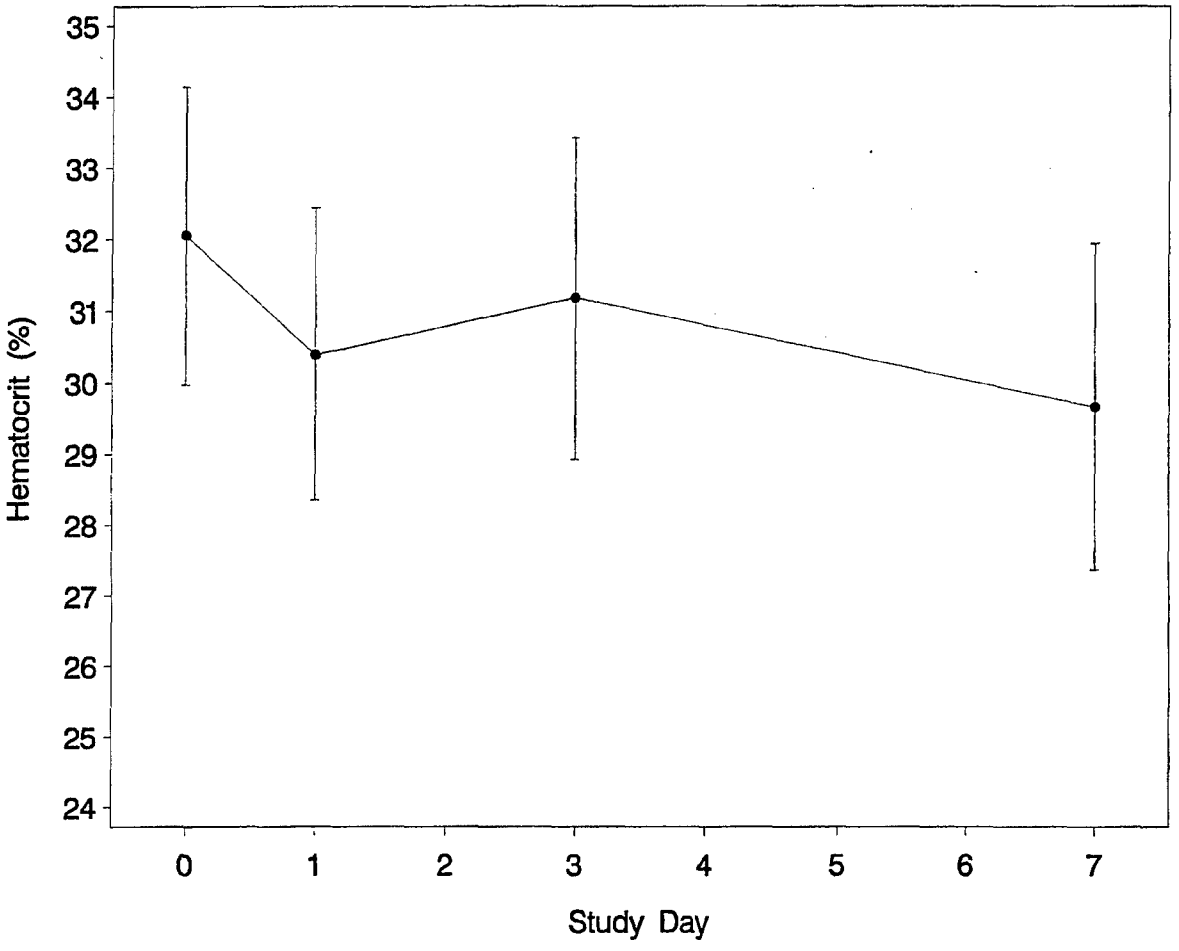

Figure A-25. Mean ± 2 Standard Errors Hematocrit (%) by Study Day for the Six Animals Tested in Phase 1, Part B.

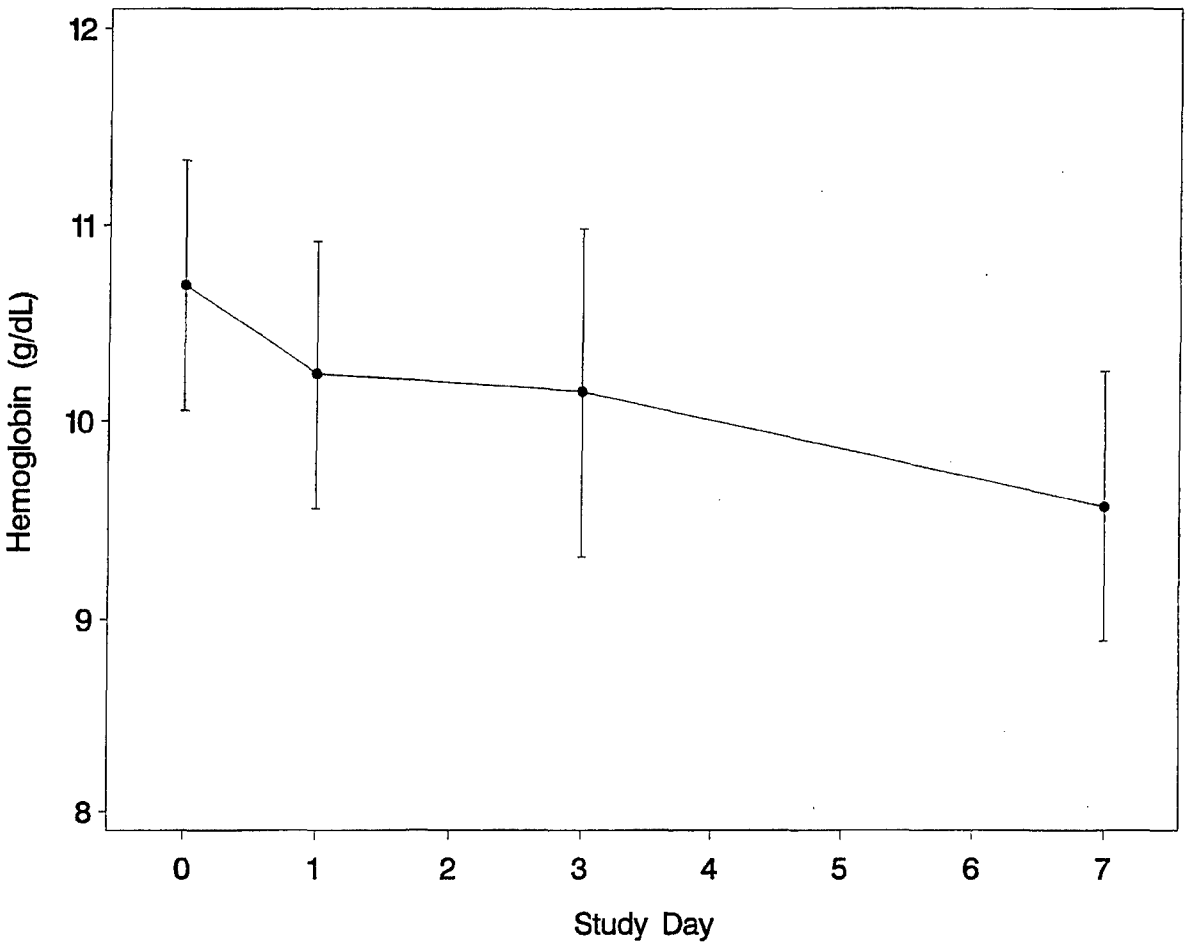

Figure A-26. Mean ± 2 Standard Errors Hemoglobin (g/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

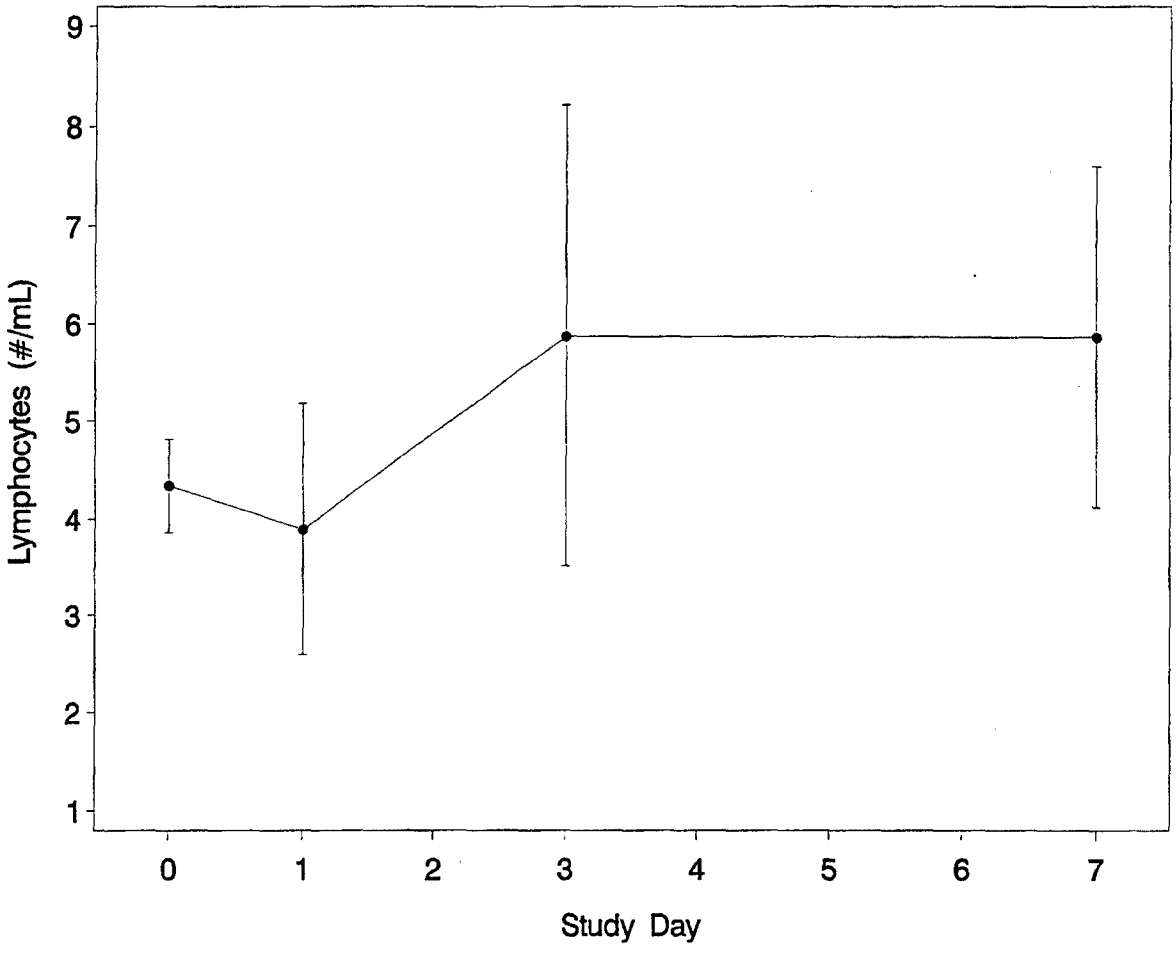

Figure A-27. Mean ± 2 Standard Errors Lymphocytes (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

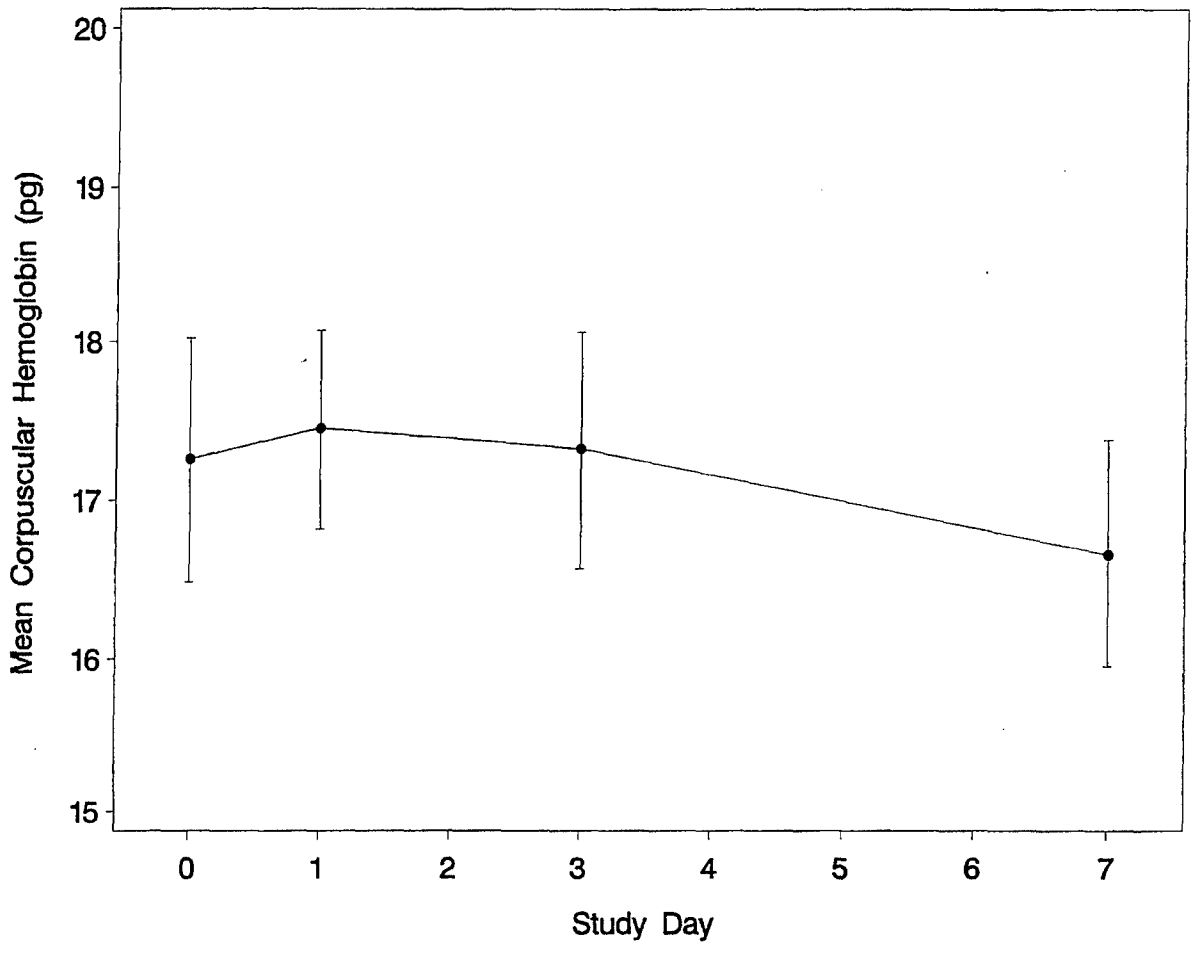

Figure A-28. Mean ± 2 Standard Errors Mean Corpuscular Hemoglobin (pg) by Study Day for the Six Animals Tested in Phase 1, Part B.

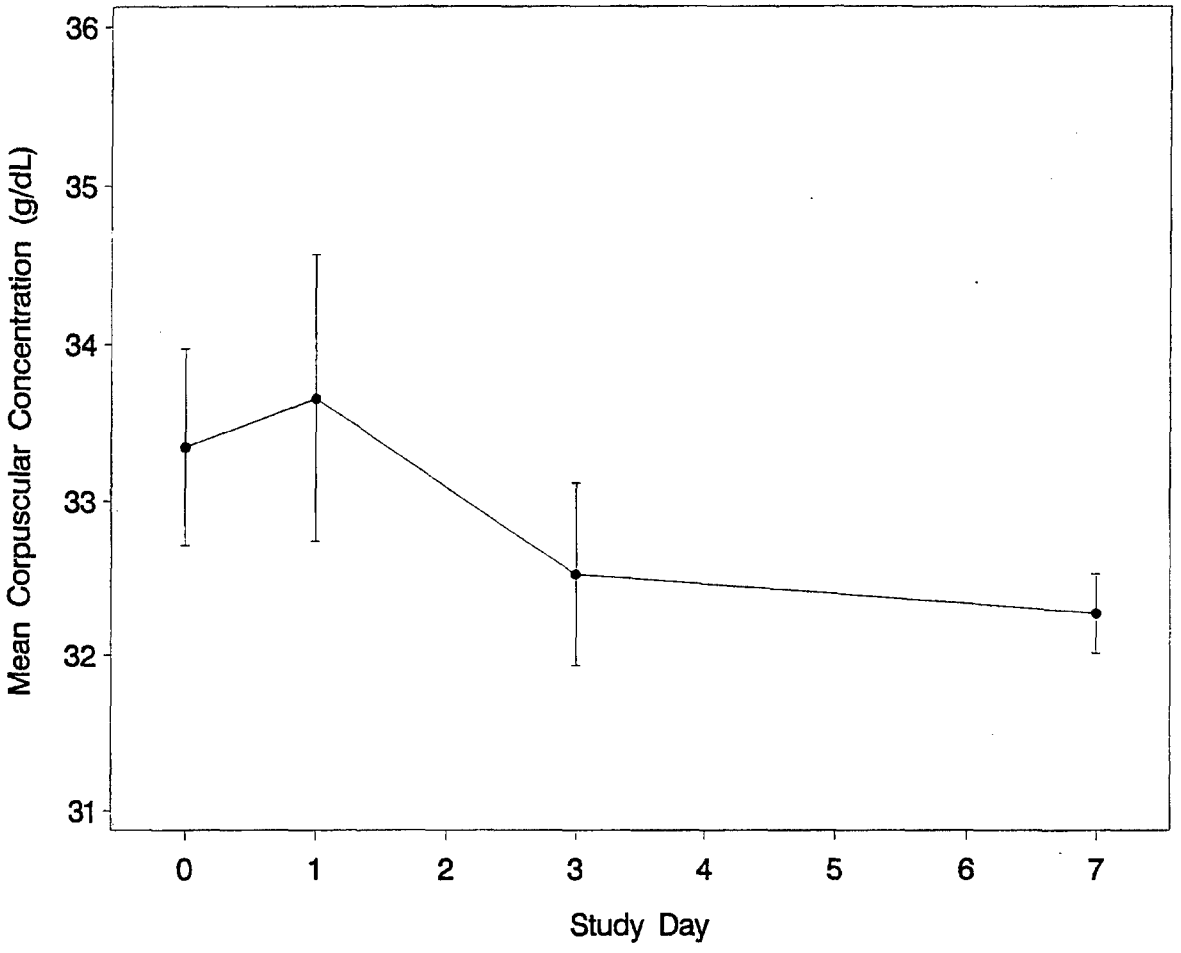

Figure A-29. Mean ± 2 Standard Errors Mean Corpuscular Concentration (g/dL) by Study Day for the Six Animals Tested in Phase 1, Part B.

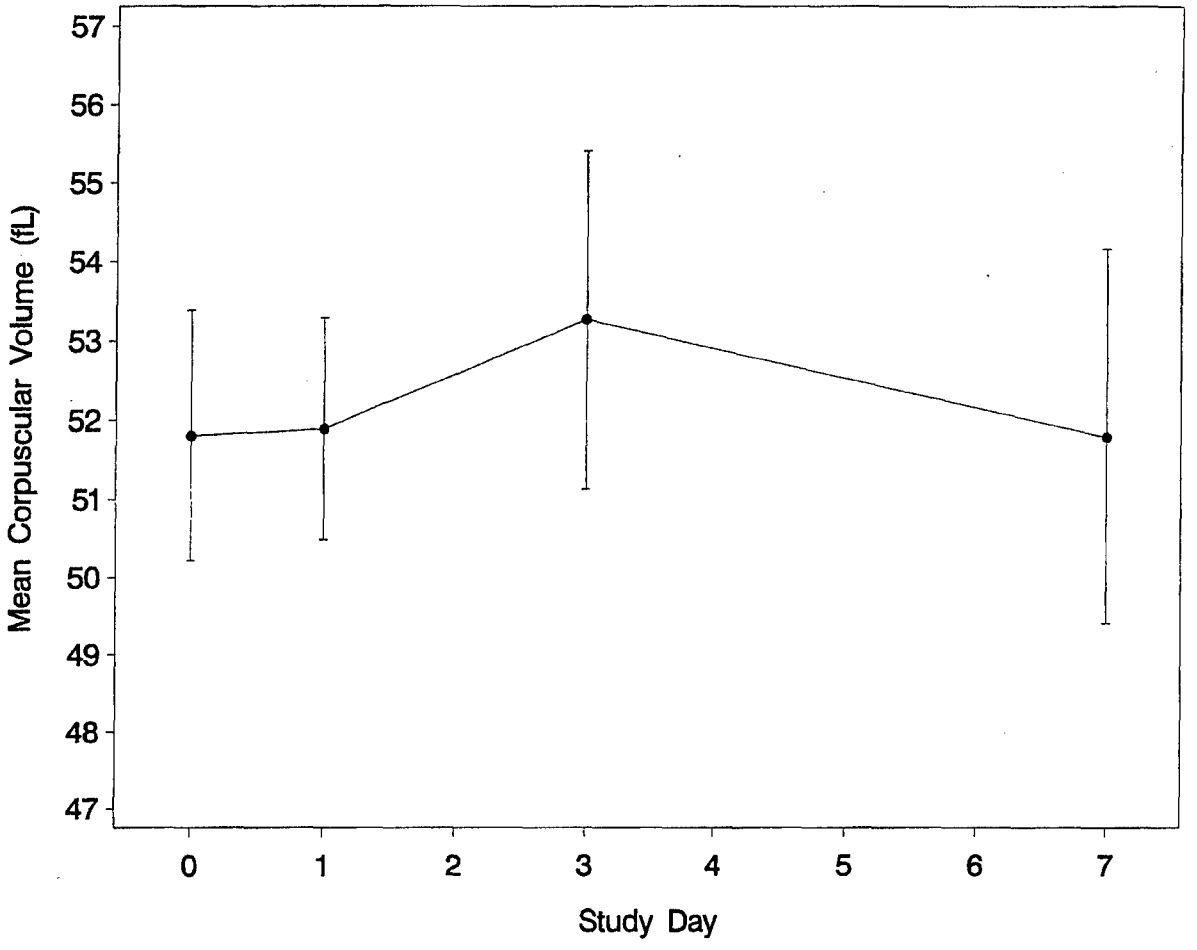

Figure A-30. Mean ± 2 Standard Errors Mean Corpuscular Volume (fL) by Study Day for the Six Animals Tested in Phase 1, Part B.

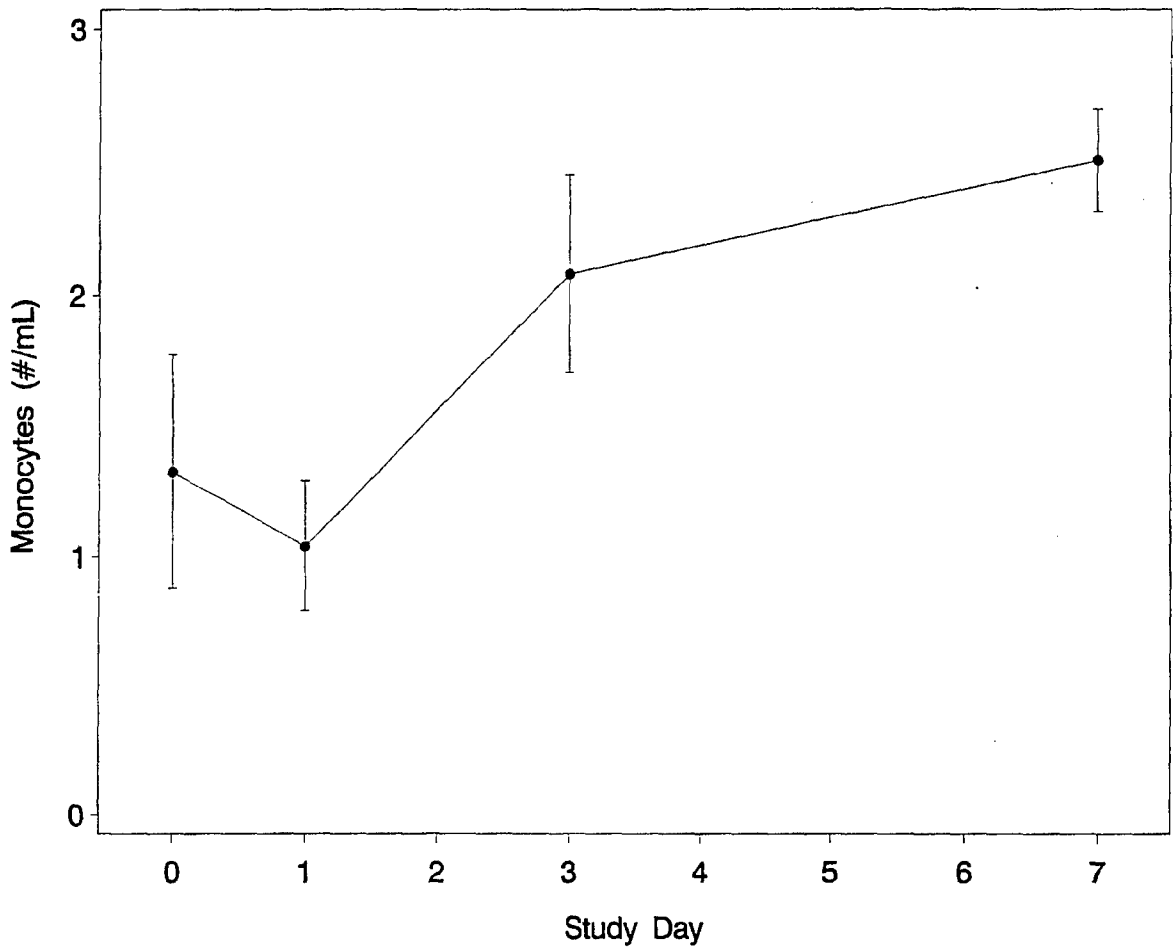

Figure A-31. Mean ± 2 Standard Errors Monocytes (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

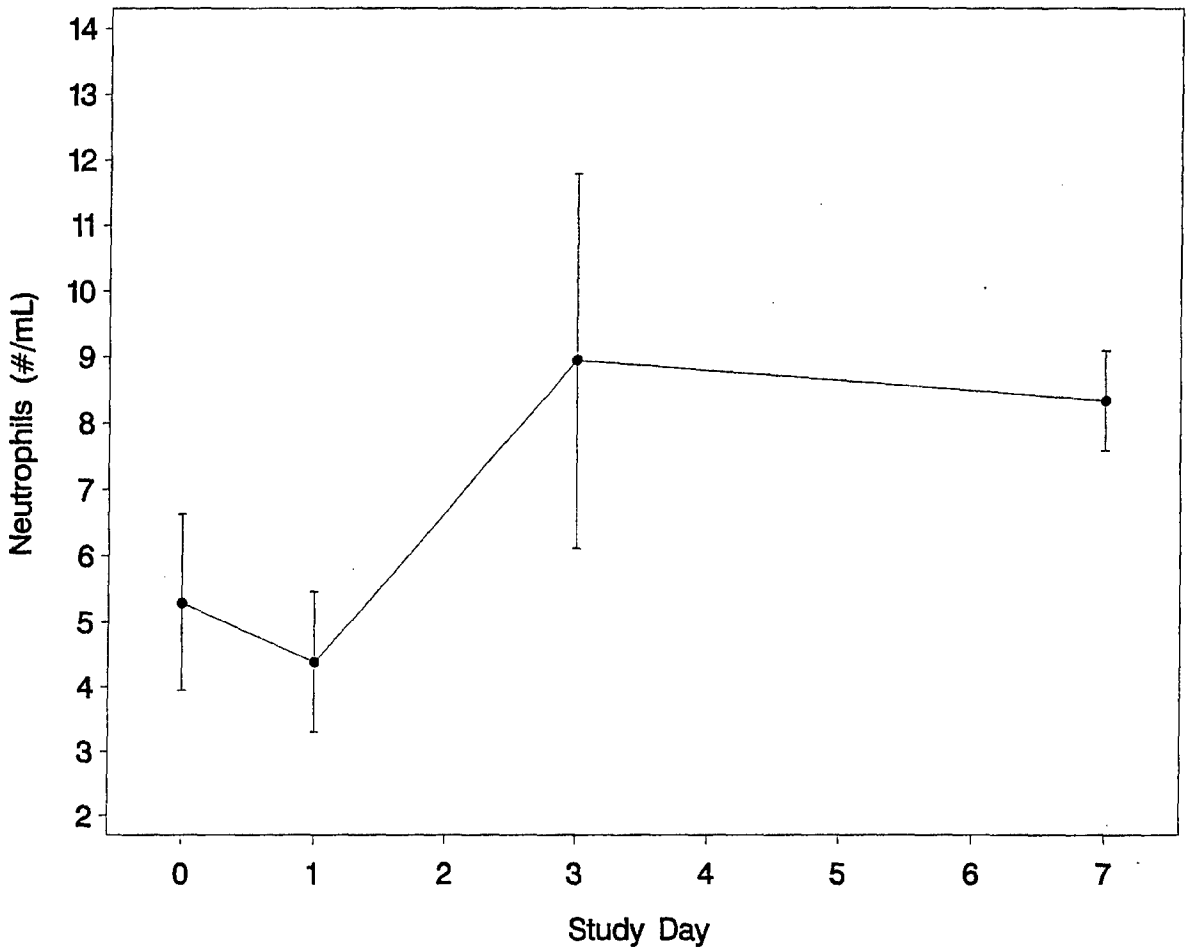

Figure A-32. Mean ± 2 Standard Errors Neutrophils (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

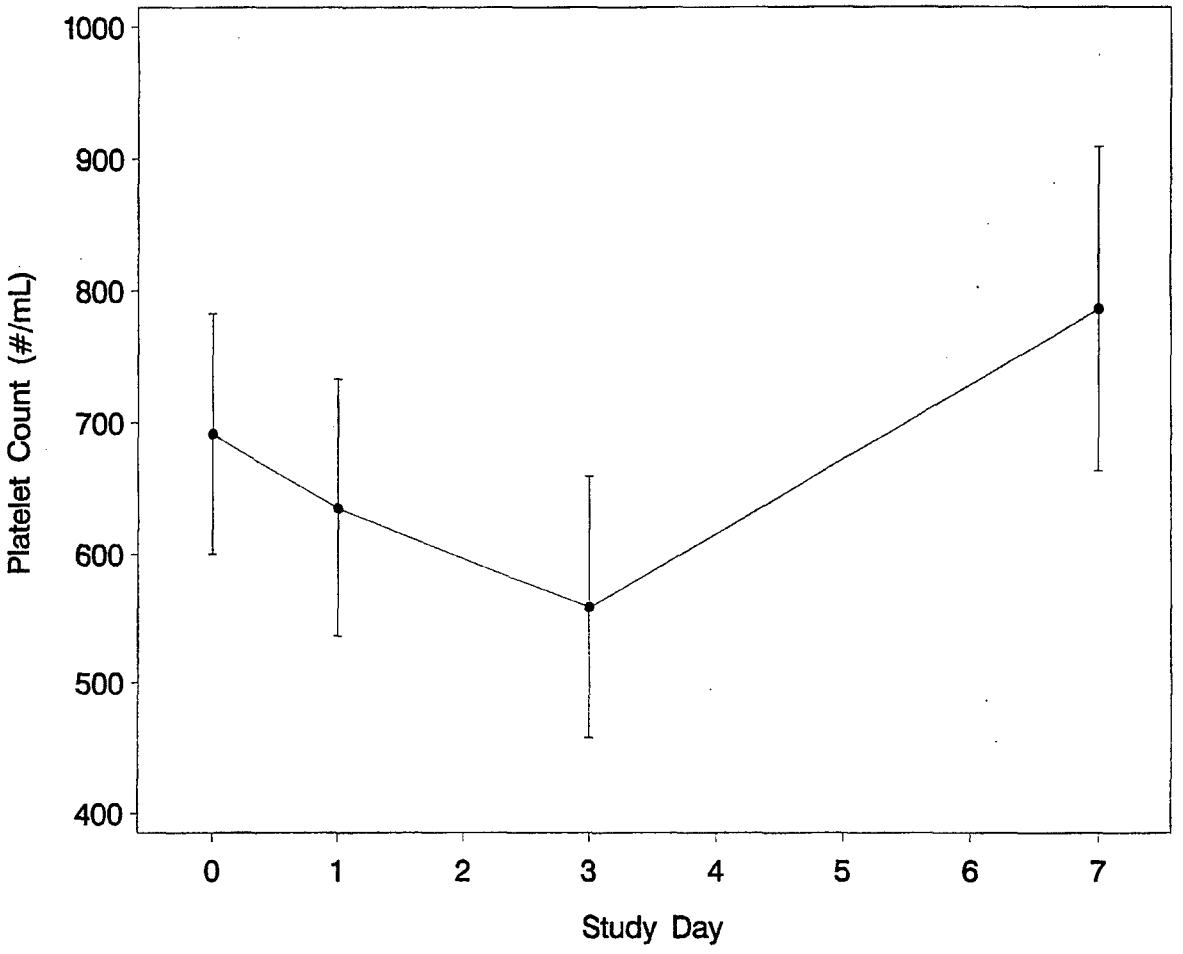

Figure A-33. Mean ± 2 Standard Errors Platelet Count (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

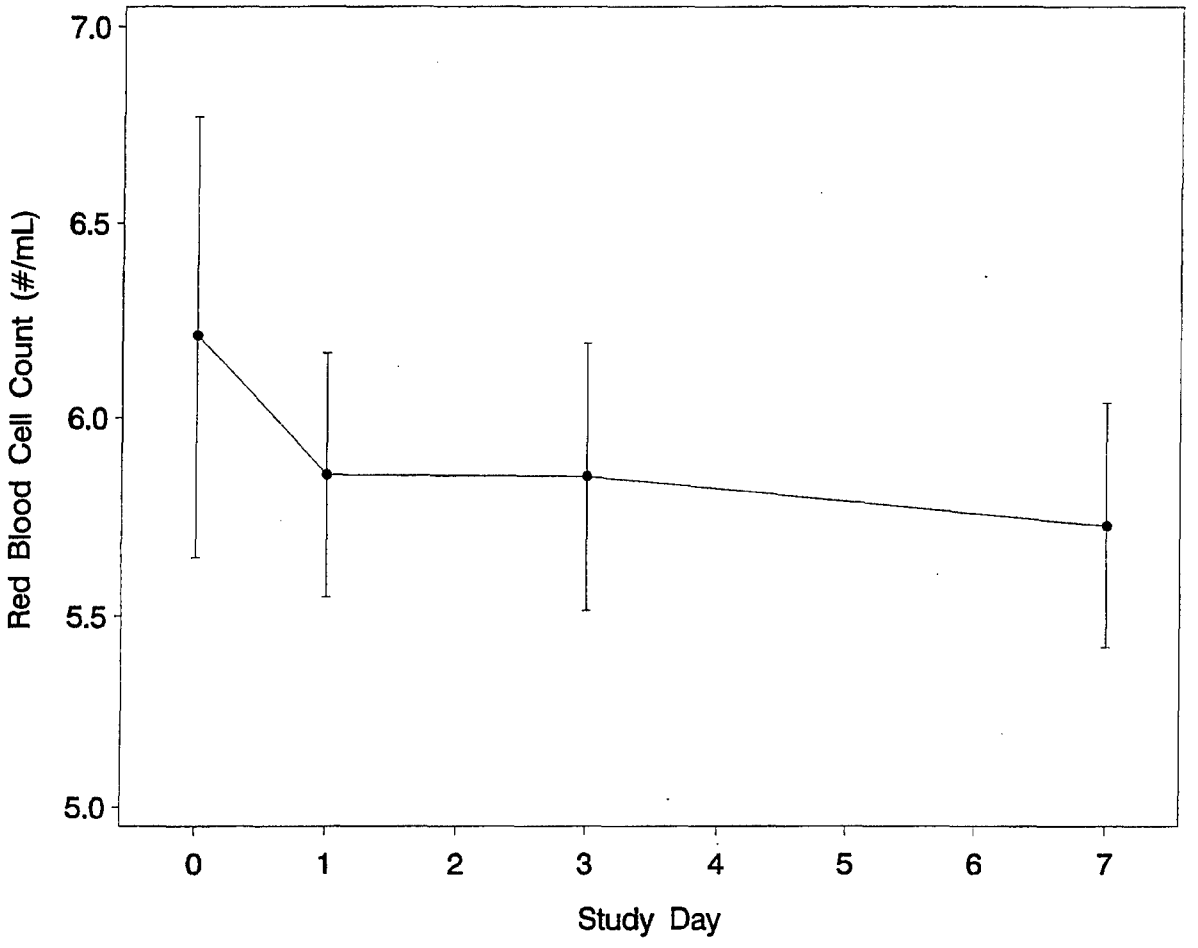

Figure A-34. Mean ± 2 Standard Errors Red Blood Cell Count (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

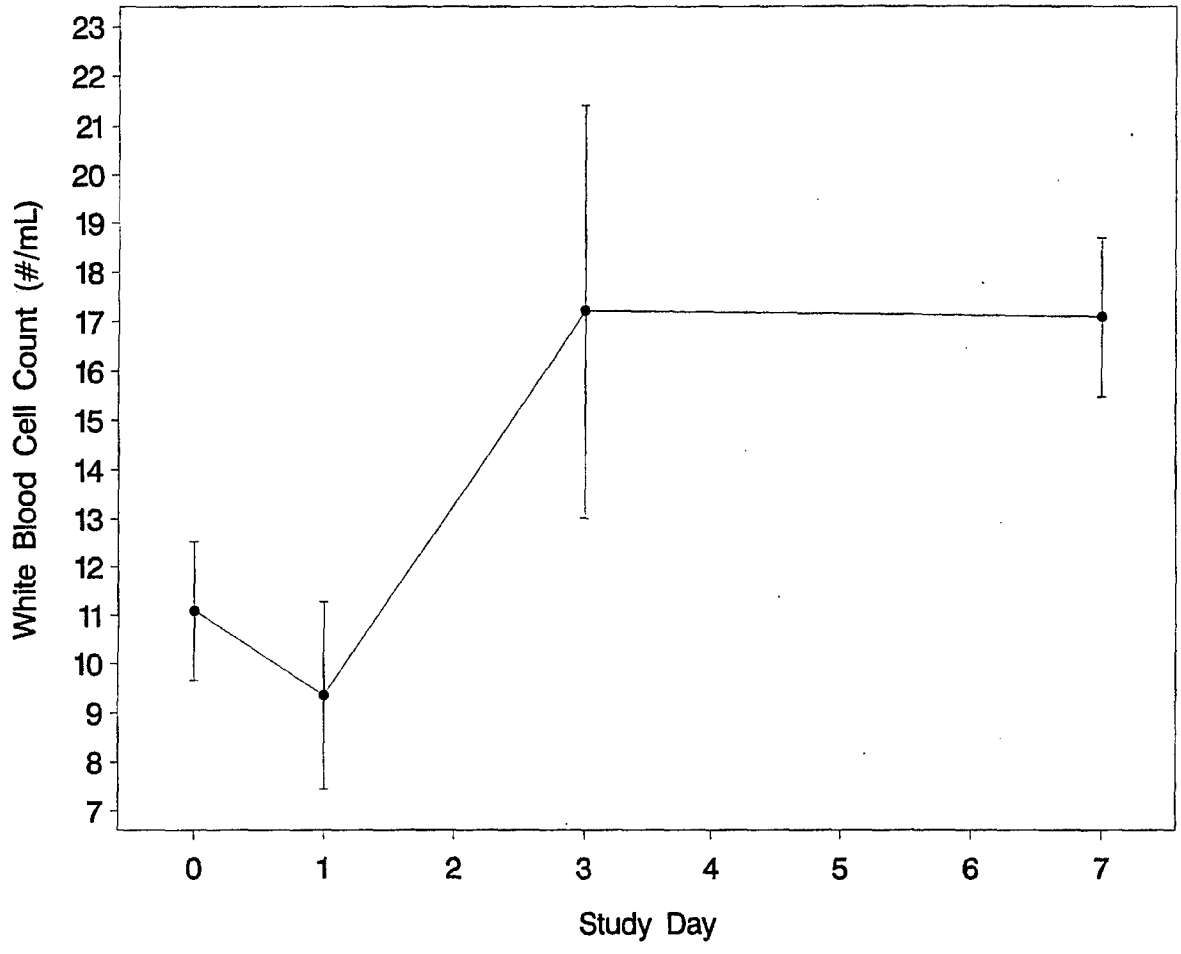

Figure A-35. Mean ± 2 Standard Errors White Blood Cell Count (#/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

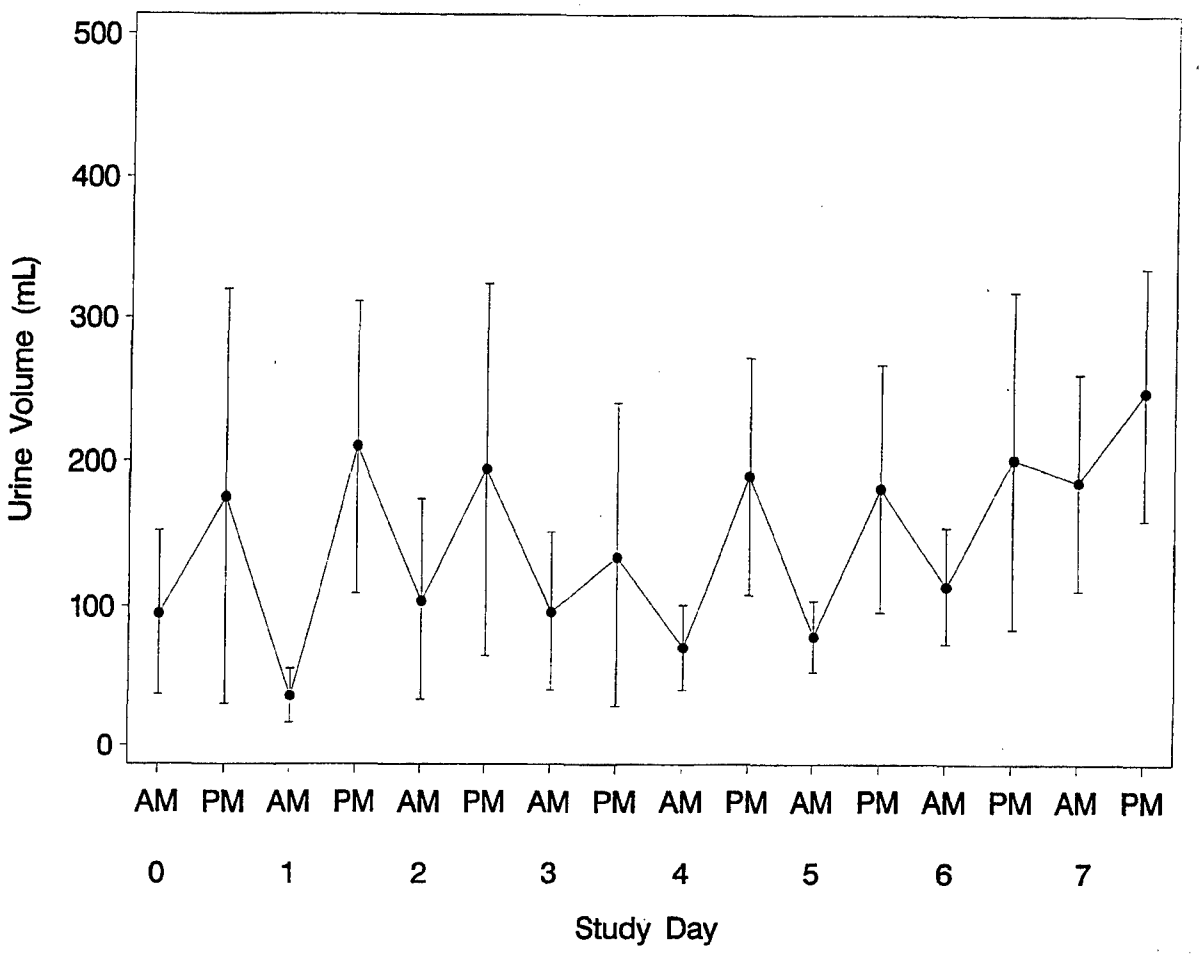

Figure A-36. Mean ± 2 Standard Errors Urine Volume (mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

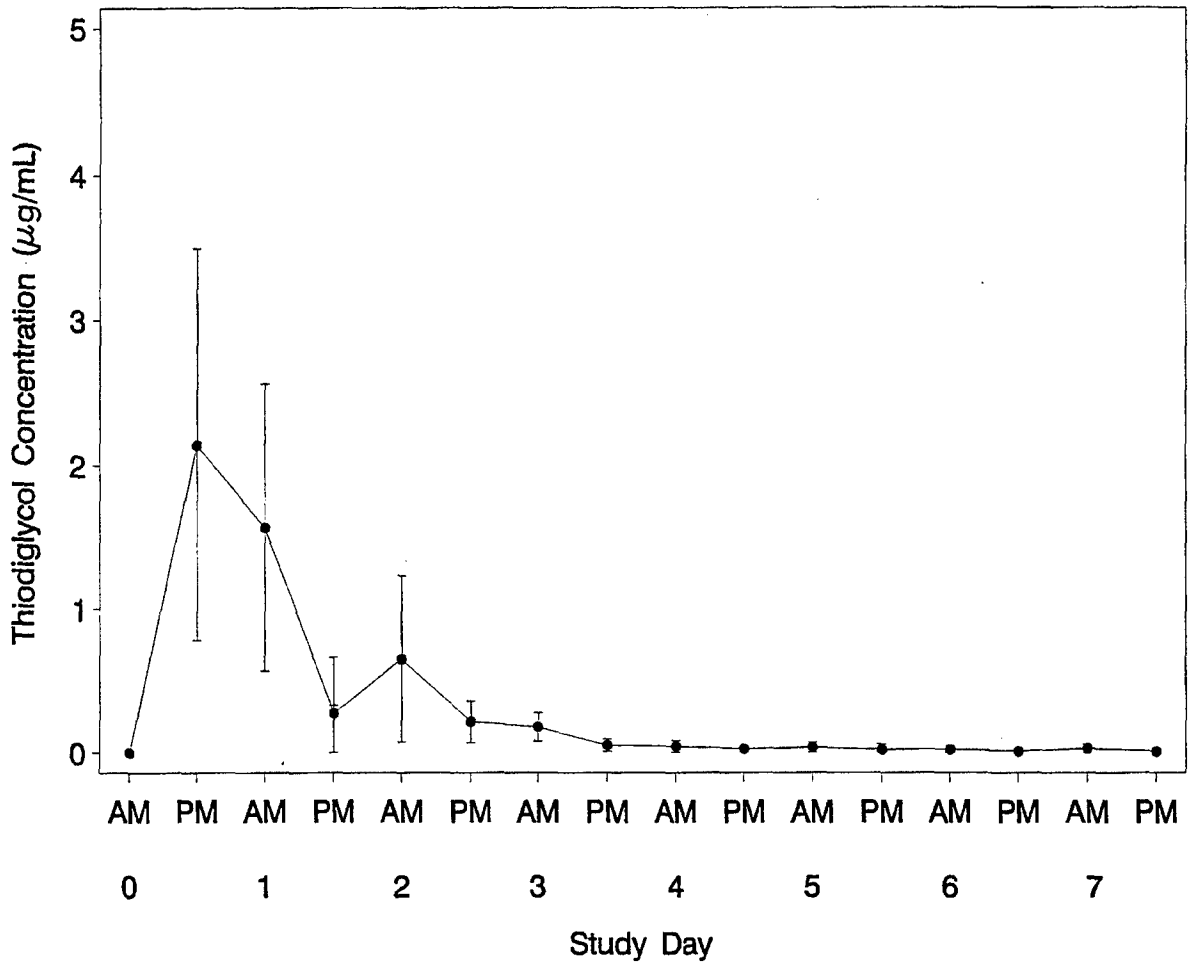

Figure A-37. Mean ± 2 Standard Errors Thiodiglycol Concentration (µg/mL) by Study Day for the Six Animals Tested in Phase 1, Part B.

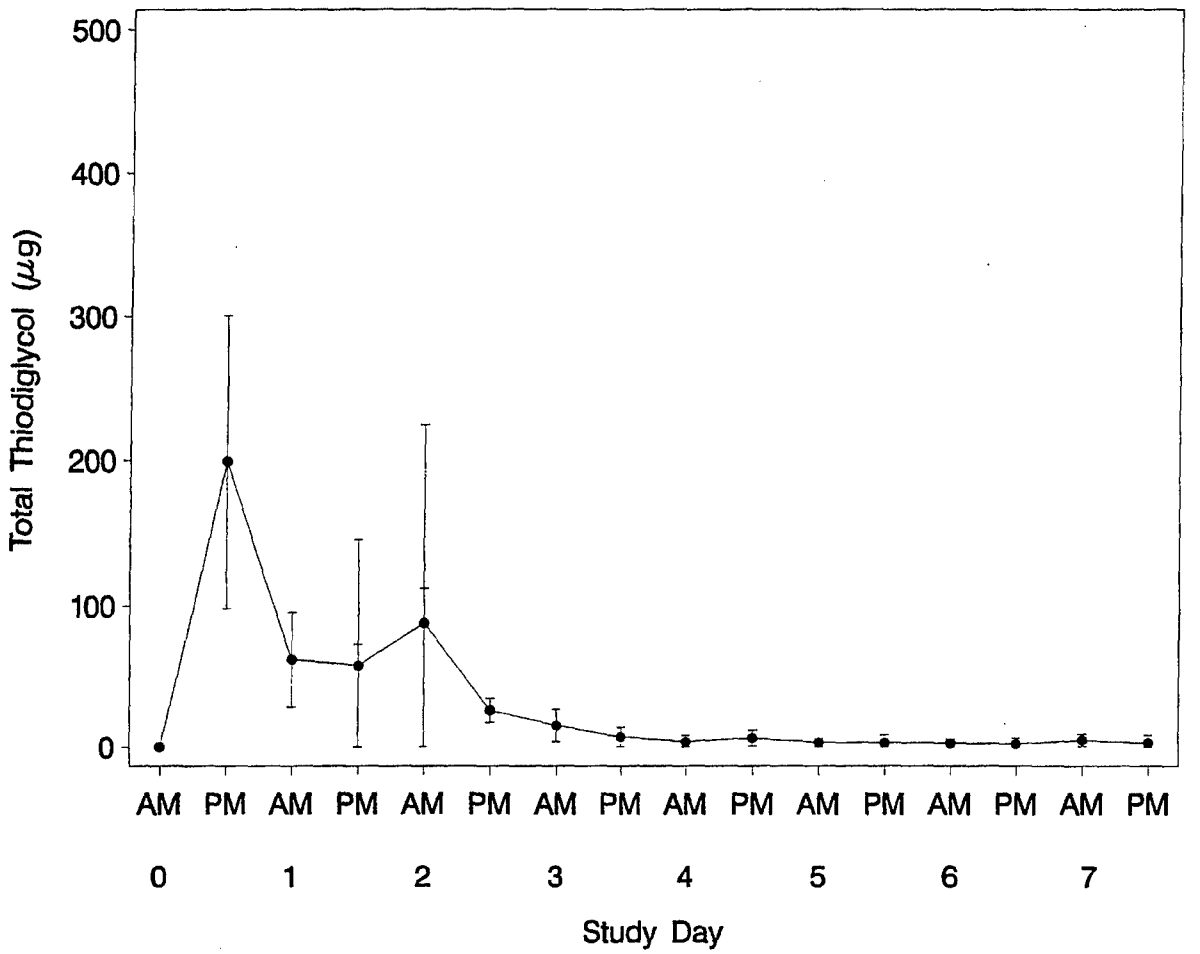

Figure A-38. Mean ± 2 Standard Errors Total Thiodiglycol (µg) by Study Day for the Six Animals Tested in Phase 1, Part B.

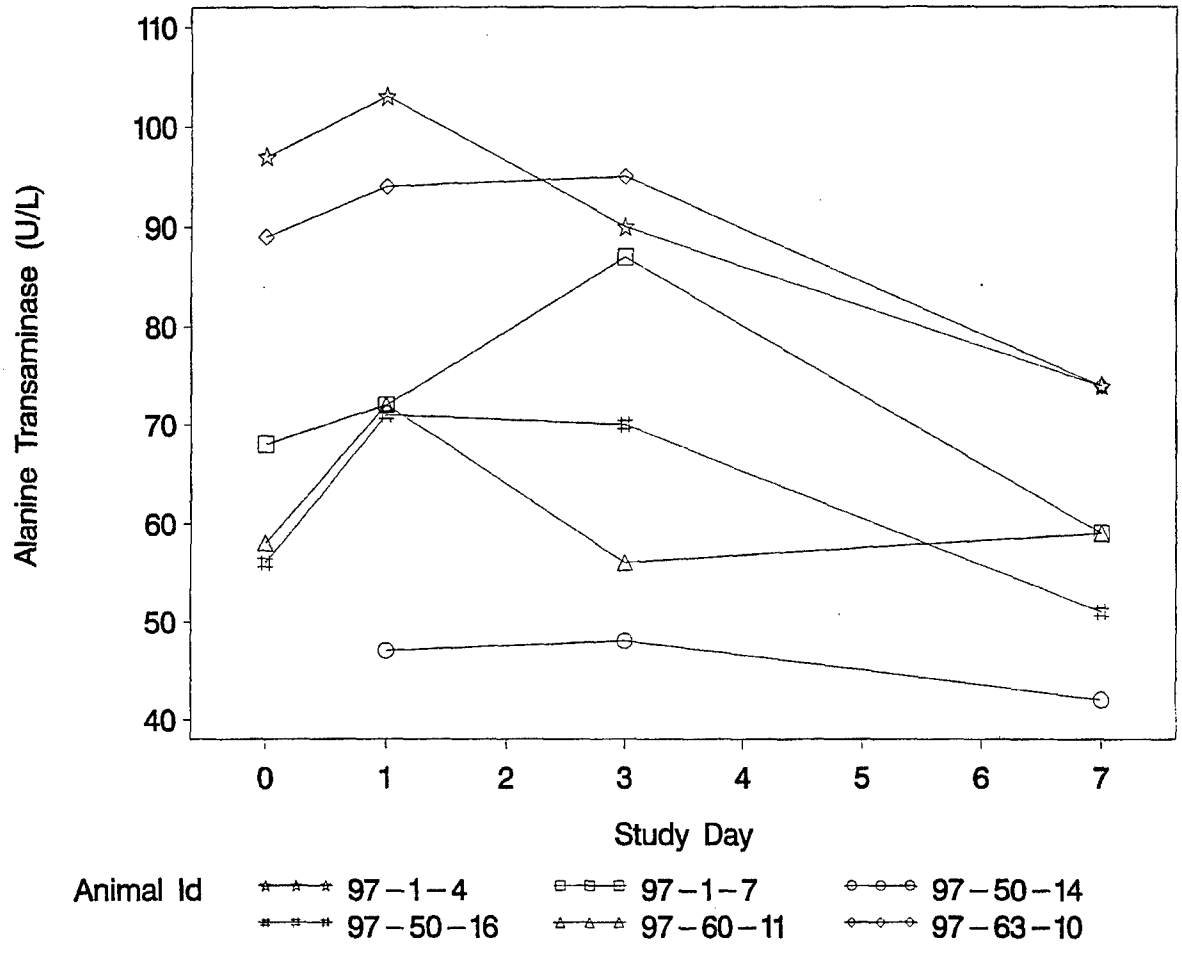

Figure B-1. Alanine Transaminase (U/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

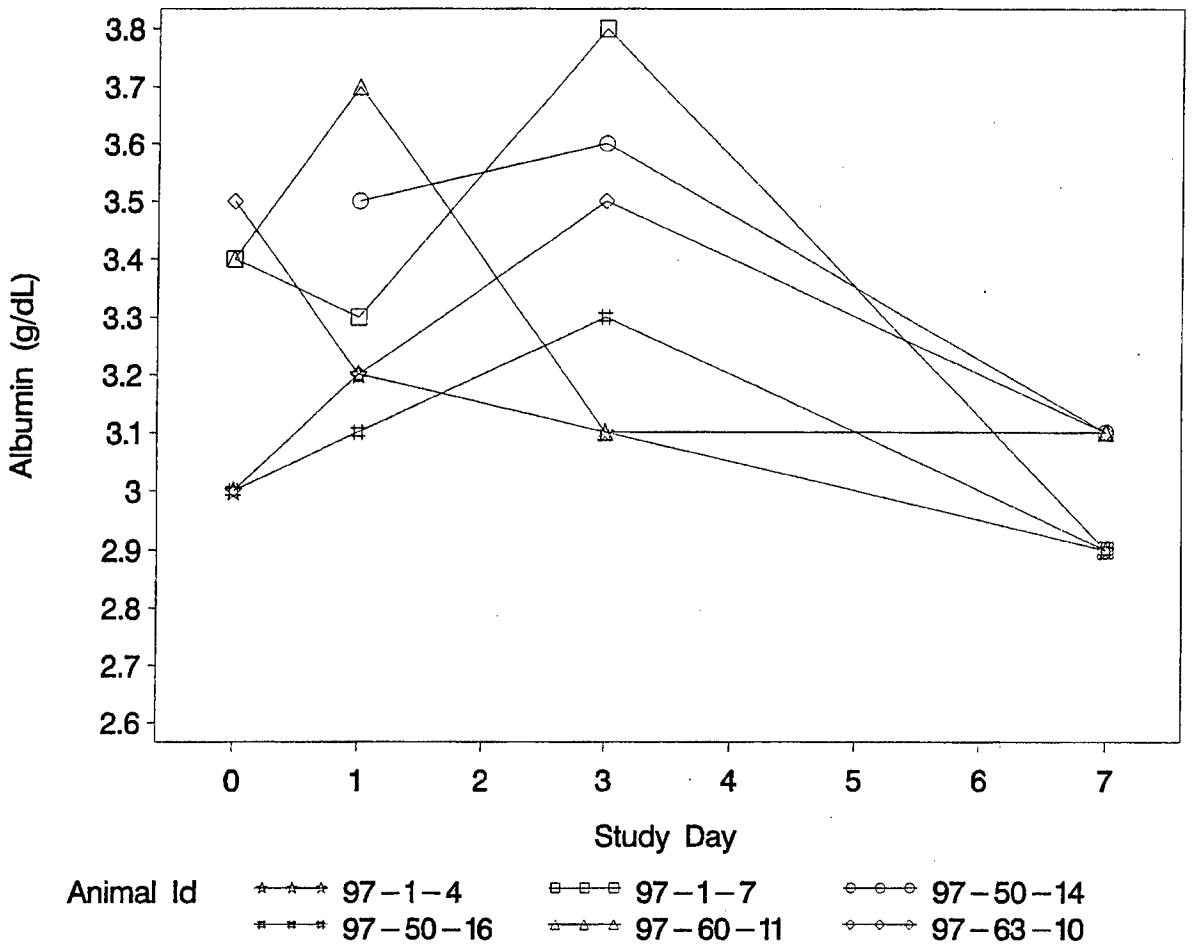

Figure B-2. Albumin (g/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

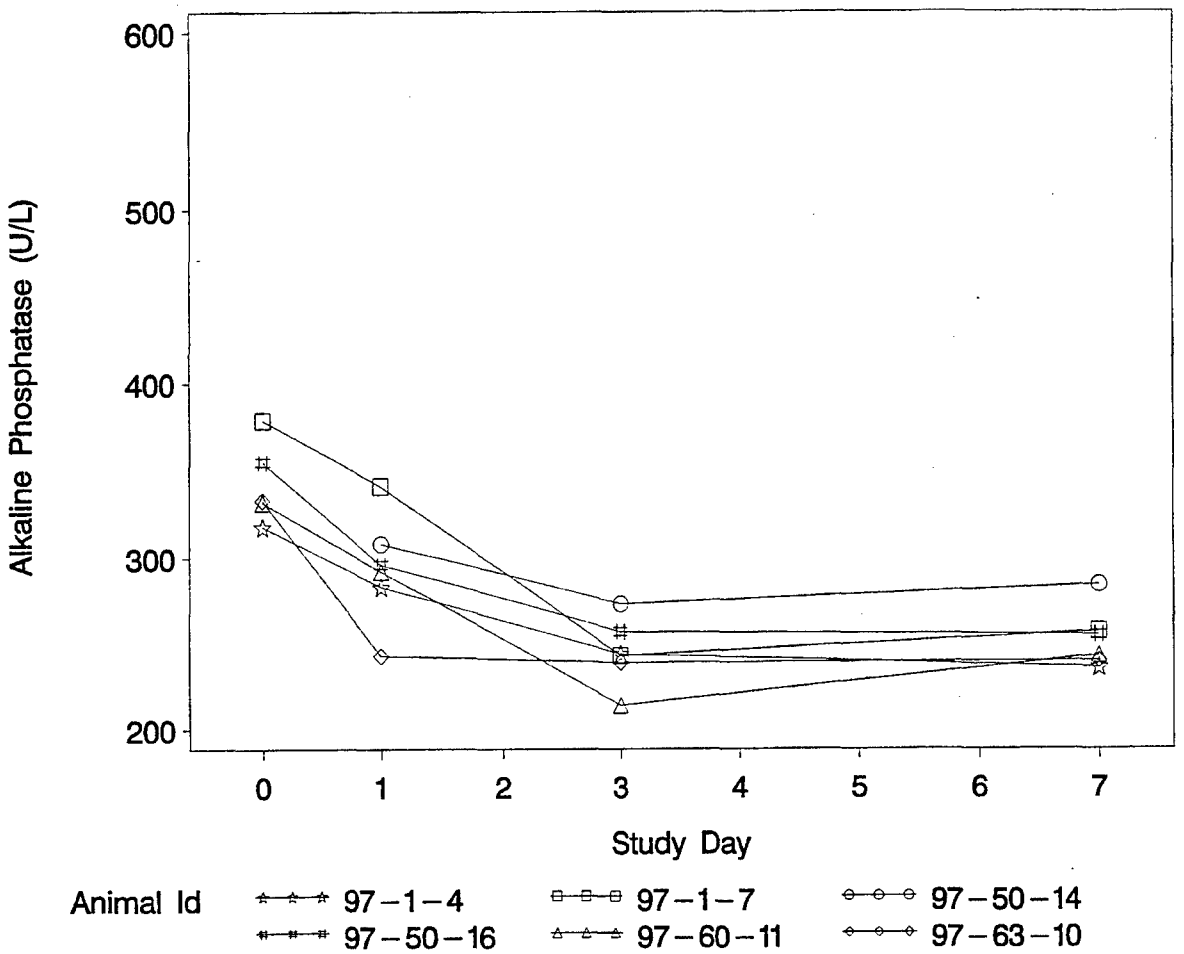

Figure B-3. Alkaline Phosphatase (U/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

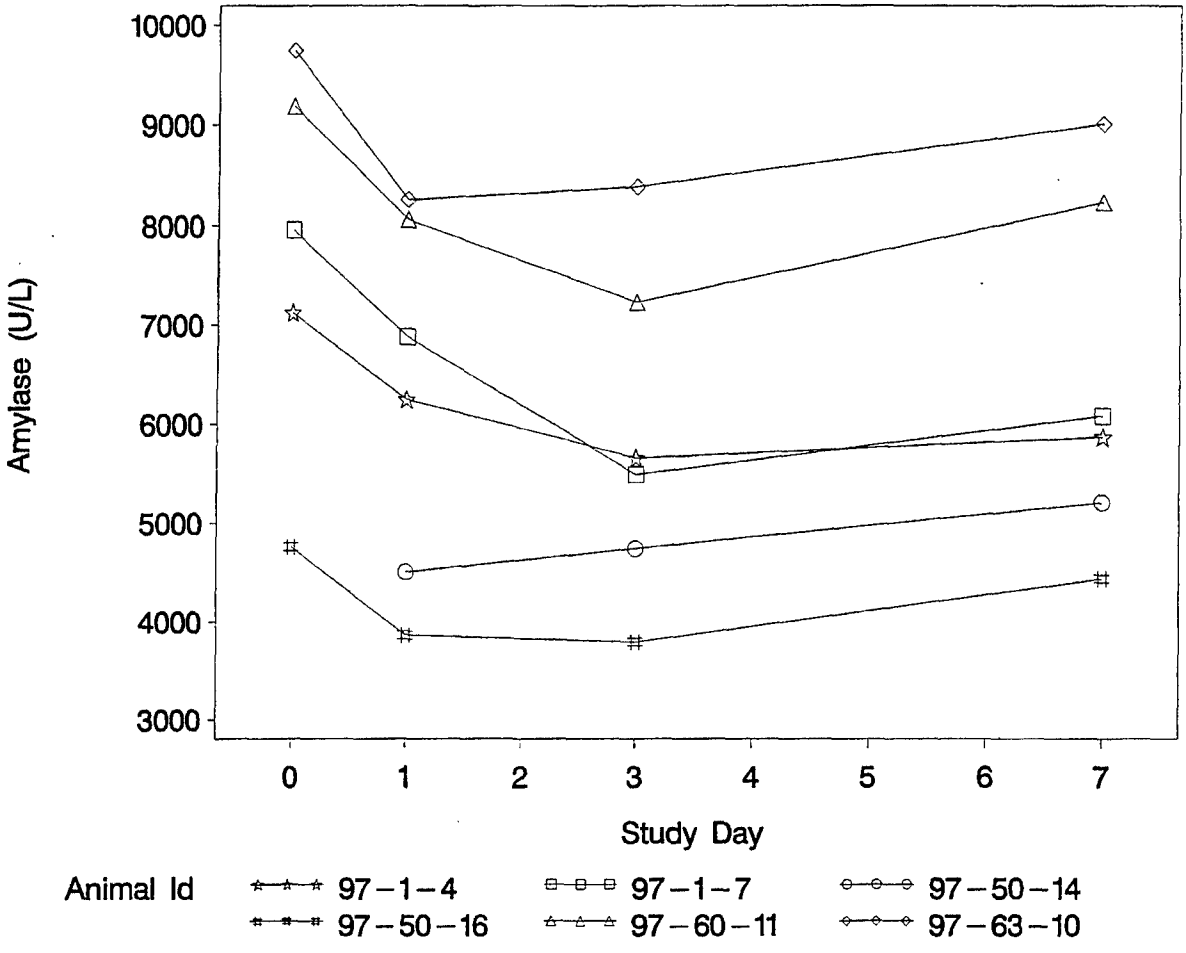

Figure B-4. Amylase (U/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

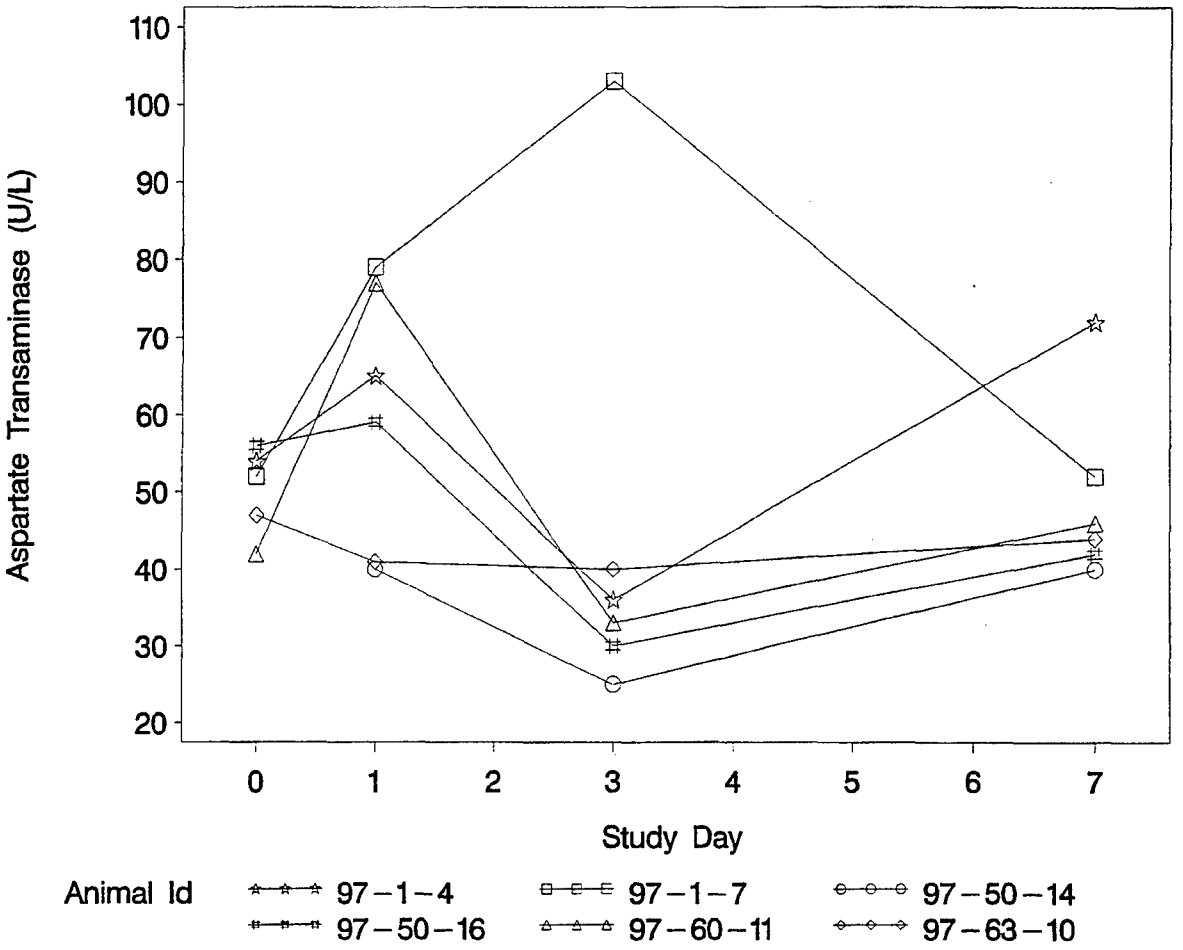

Figure B-5. Aspartate Transaminase (U/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

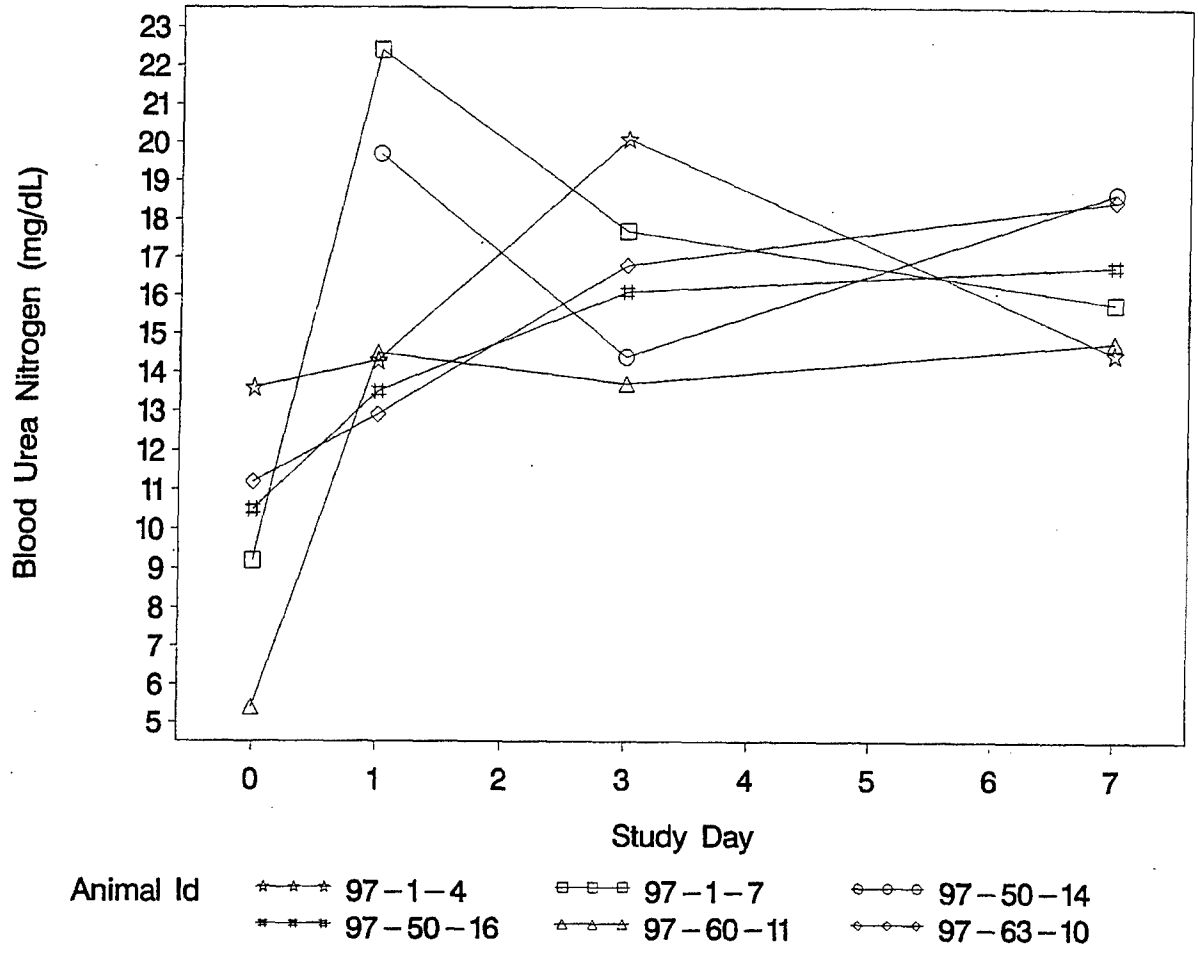

Figure B-6. Blood Urea Nitrogen (mg/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

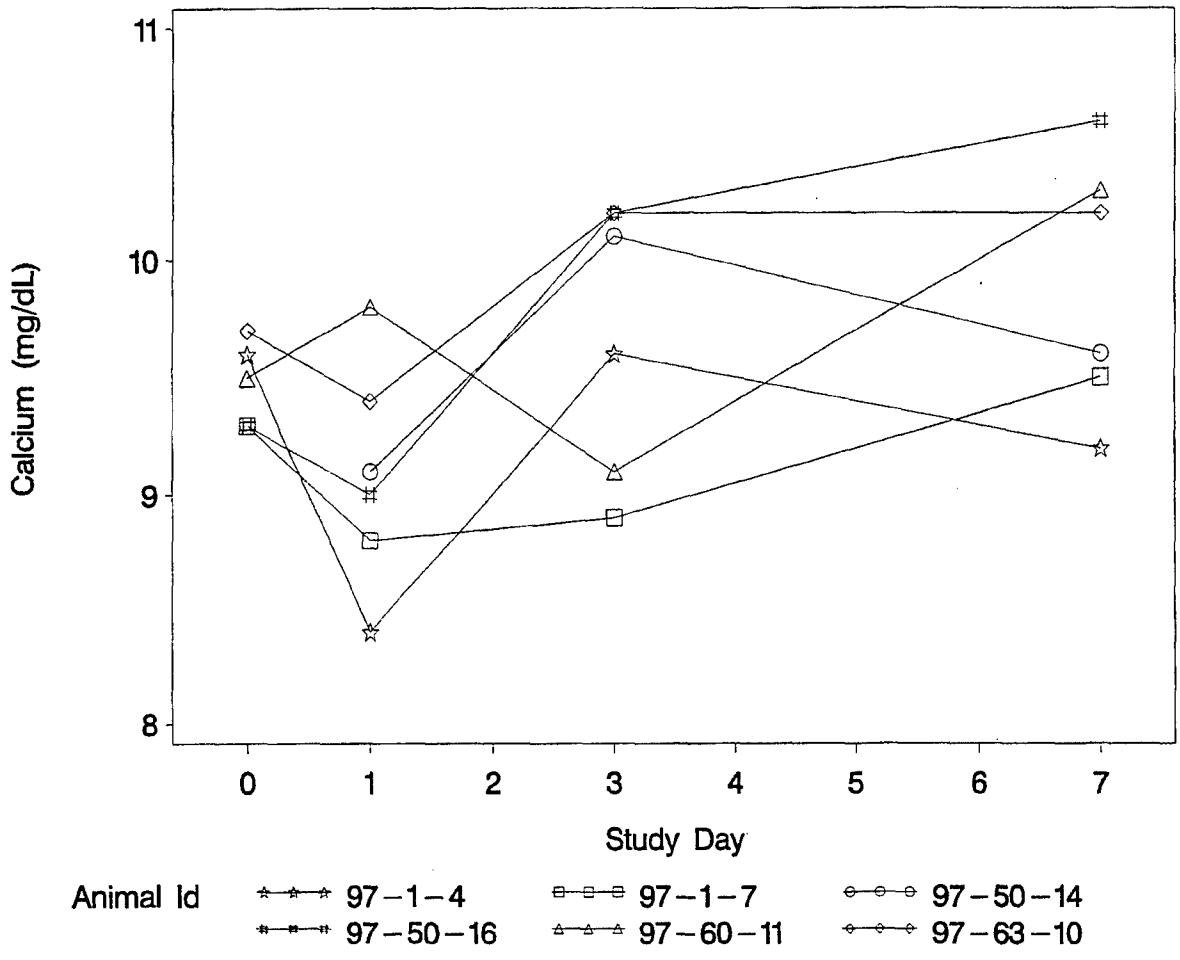

Figure B-7. Calcium (mg/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

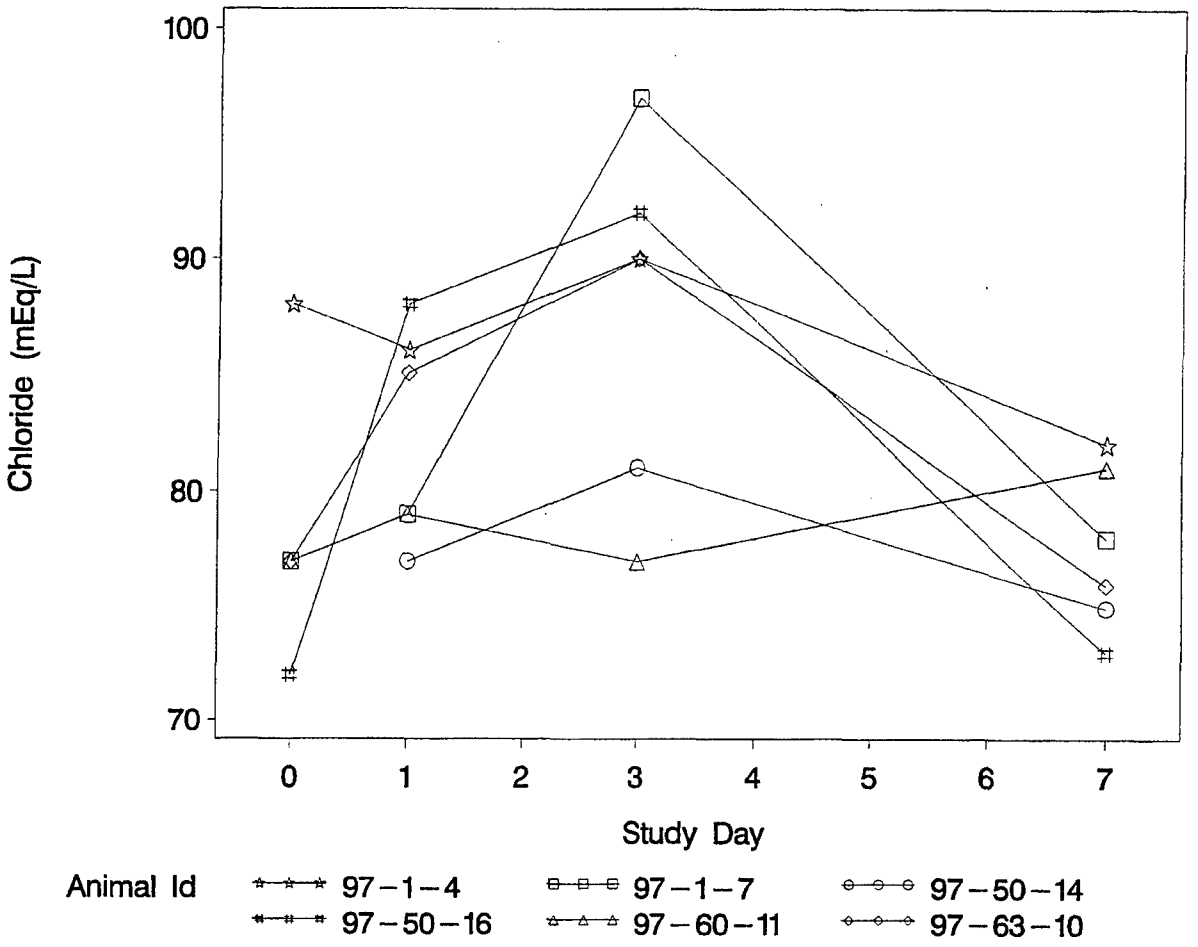

Figure B-8. Chloride (mEq/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

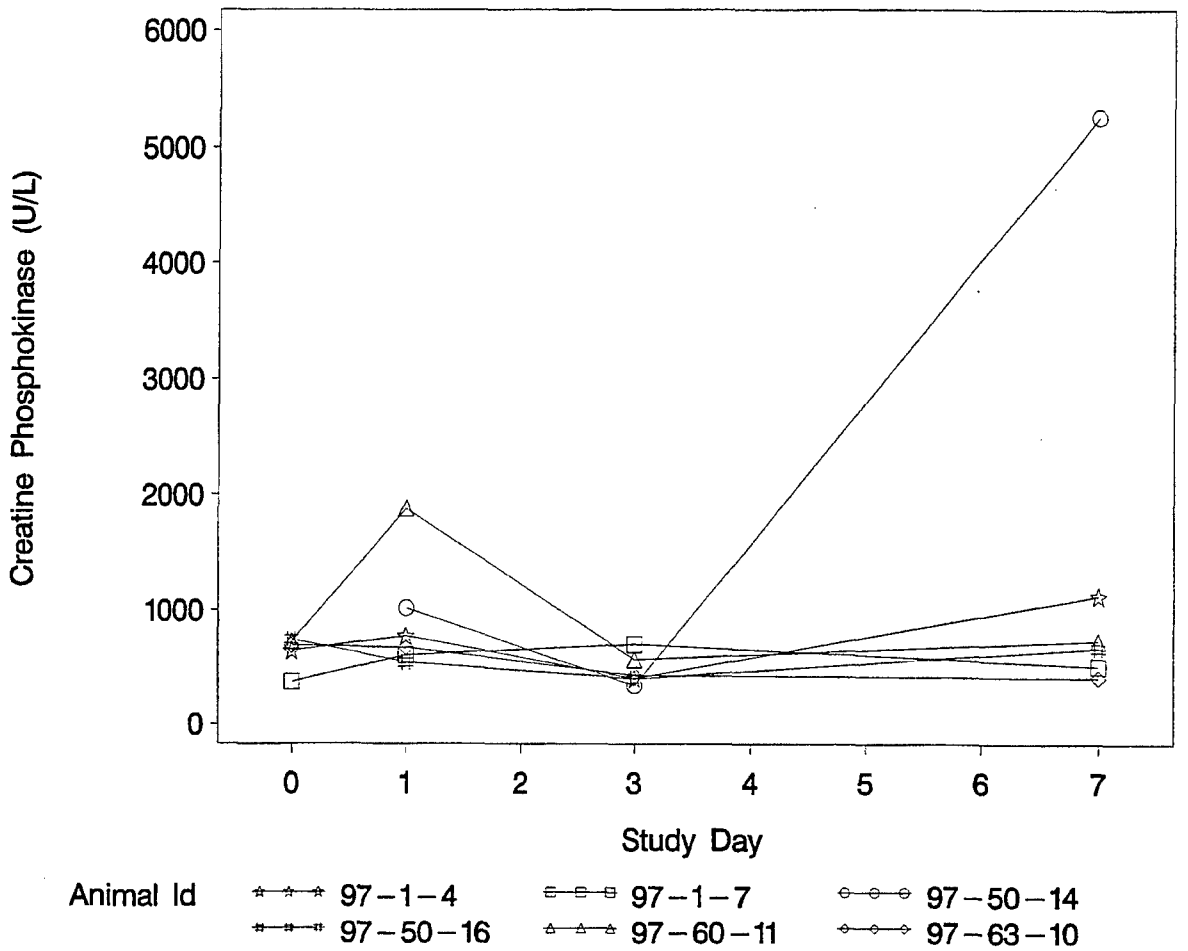

Figure B-9. Creatine Phosphokinase (U/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

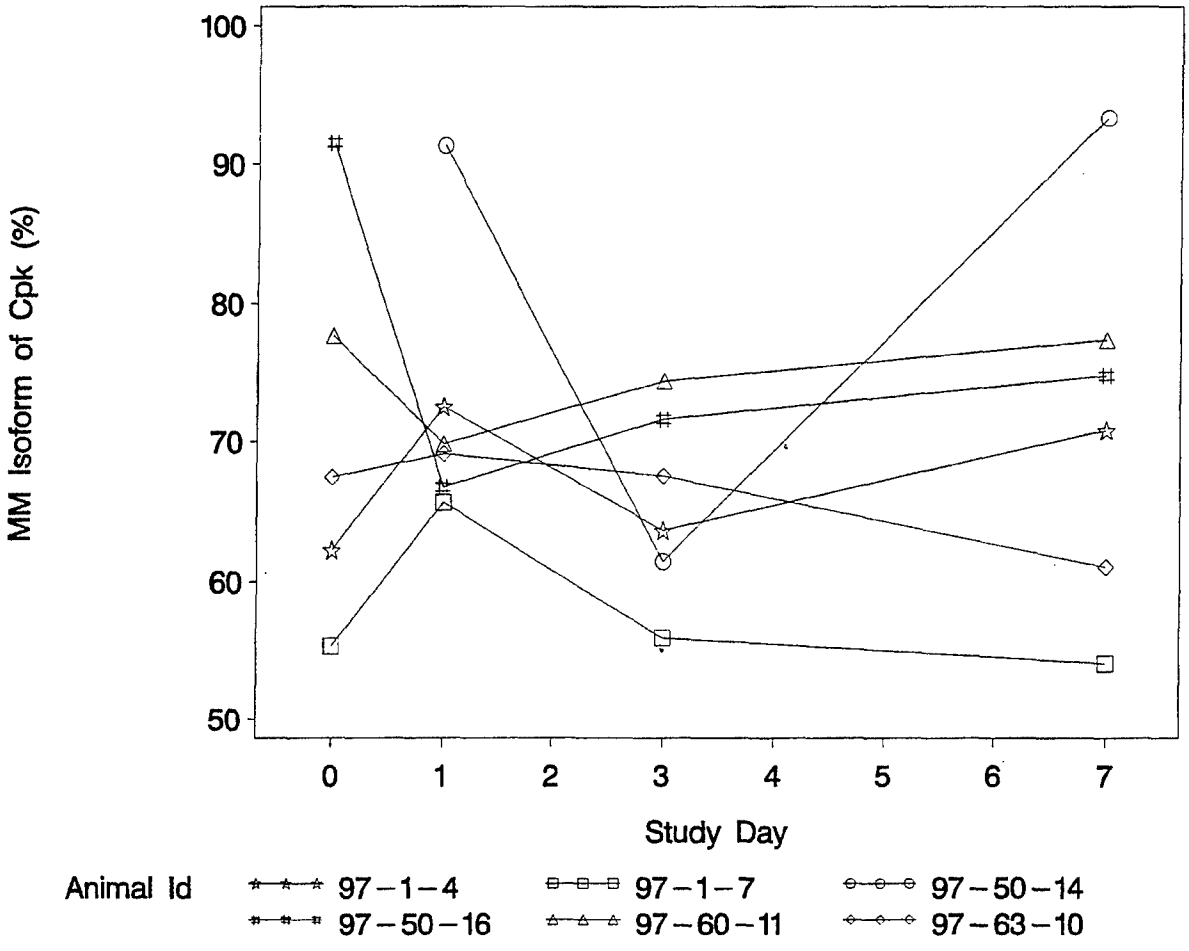

Figure B-10. MM Isoform of Creatine Phosphokinase (%) by Study Day of Six Different Animals Tested in Phase 1, Part B.

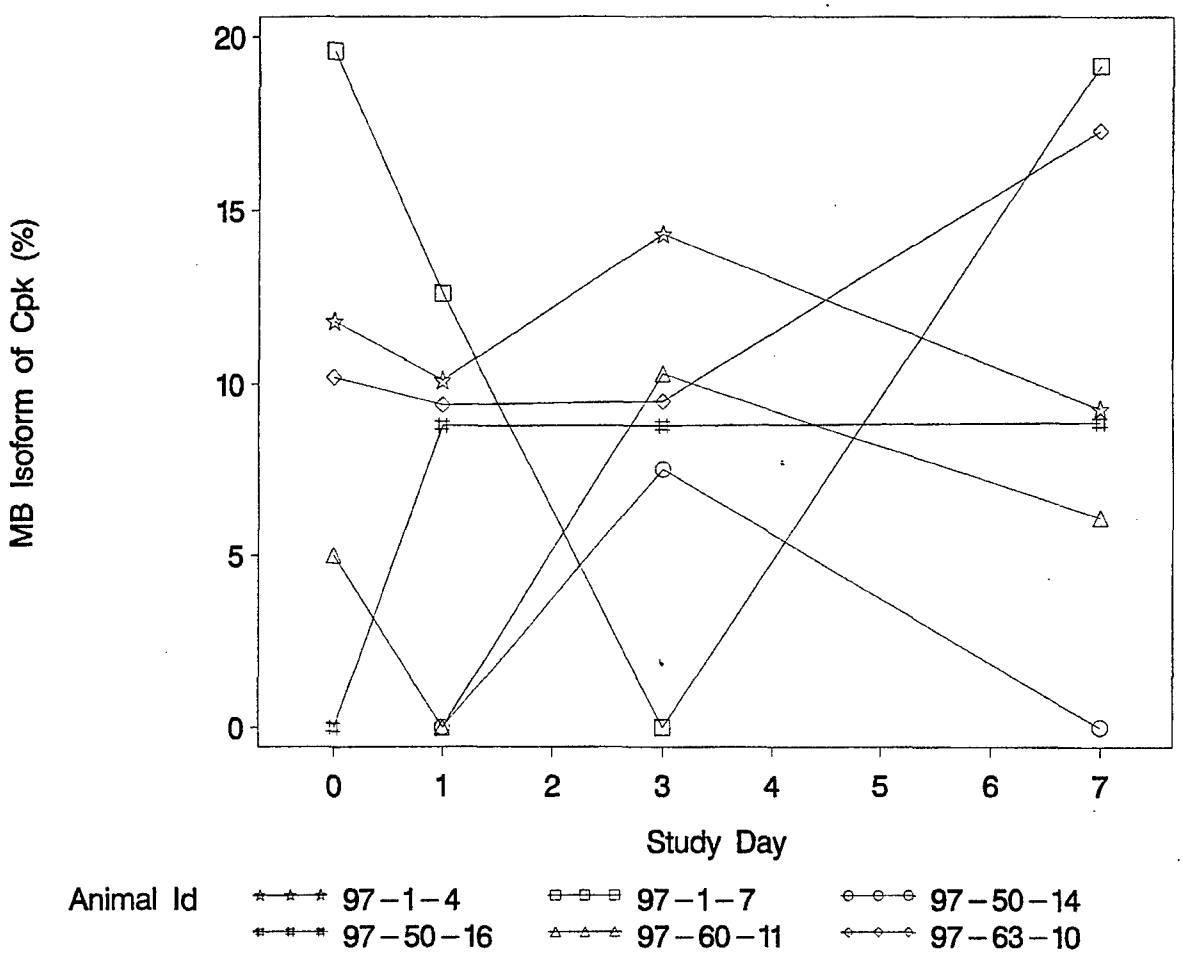

Figure B-11. MB Isoform of Creatine Phosphokinase (%) by Study Day of Six Different Animals Tested in Phase 1, Part B.

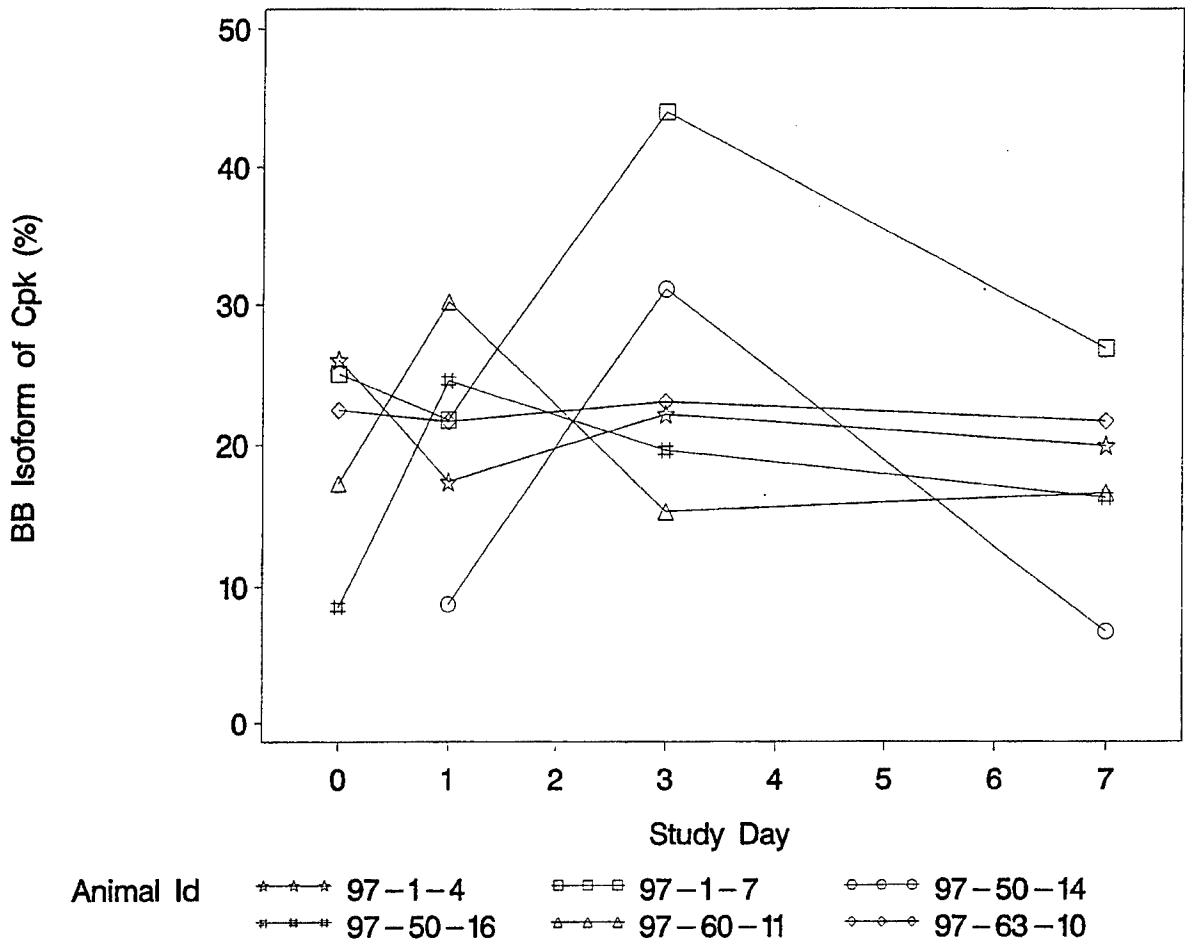

Figure B-12. BB Isoform of Creatine Phosphokinase (%) by Study Day of Six Different Animals Tested in Phase 1, Part B.

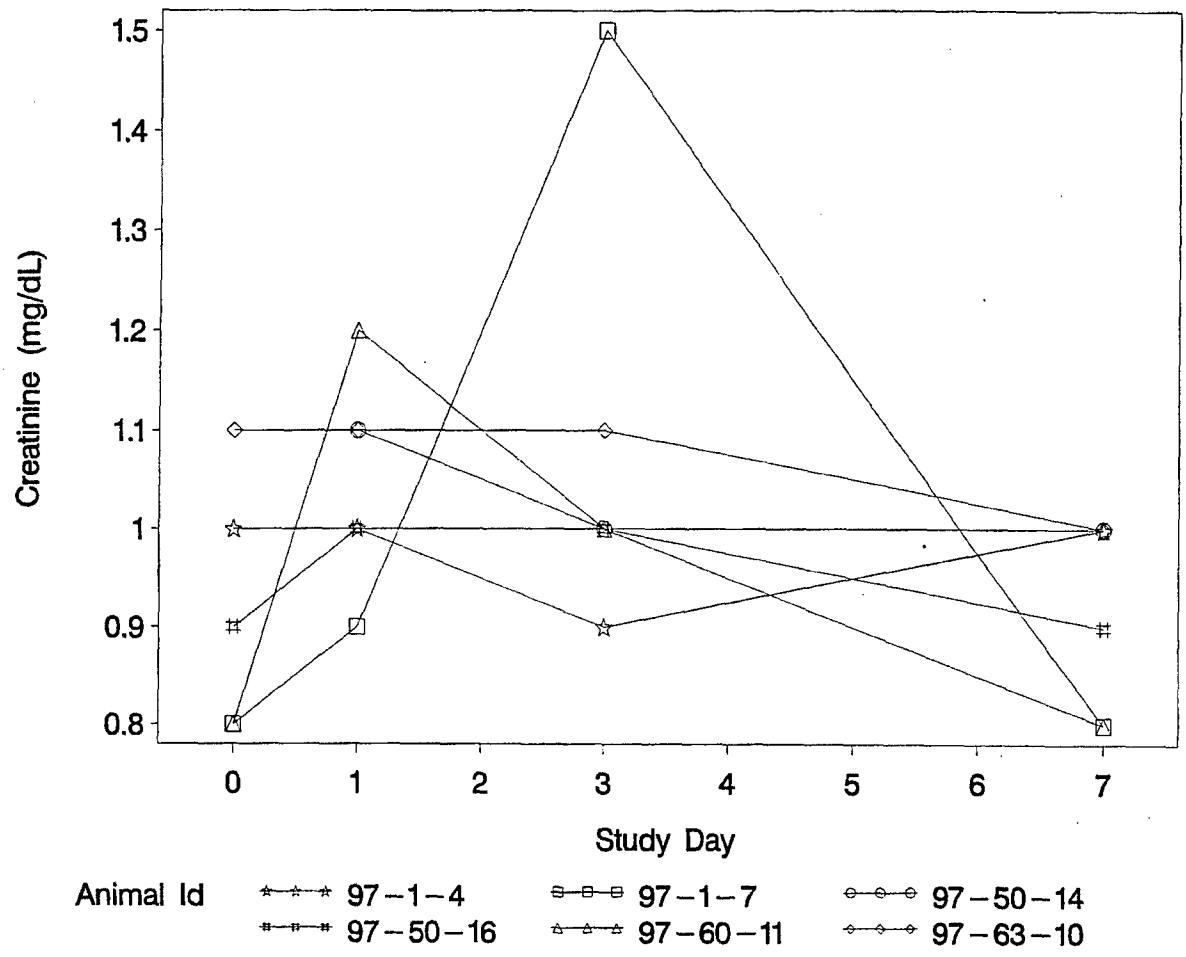

Figure B-13. Creatinine (mg/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

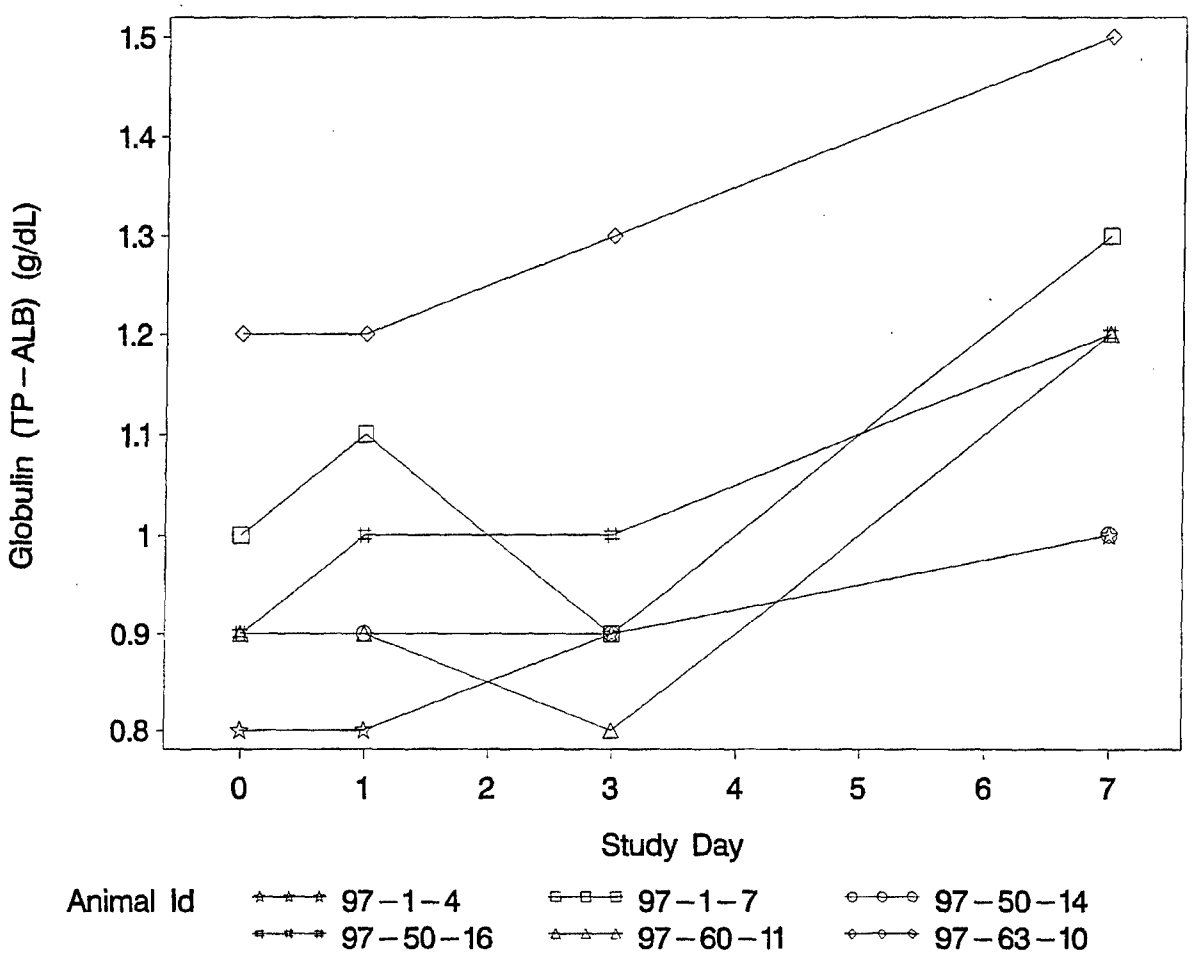

Figure B-14. Globulin (TP-ALB) (g/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

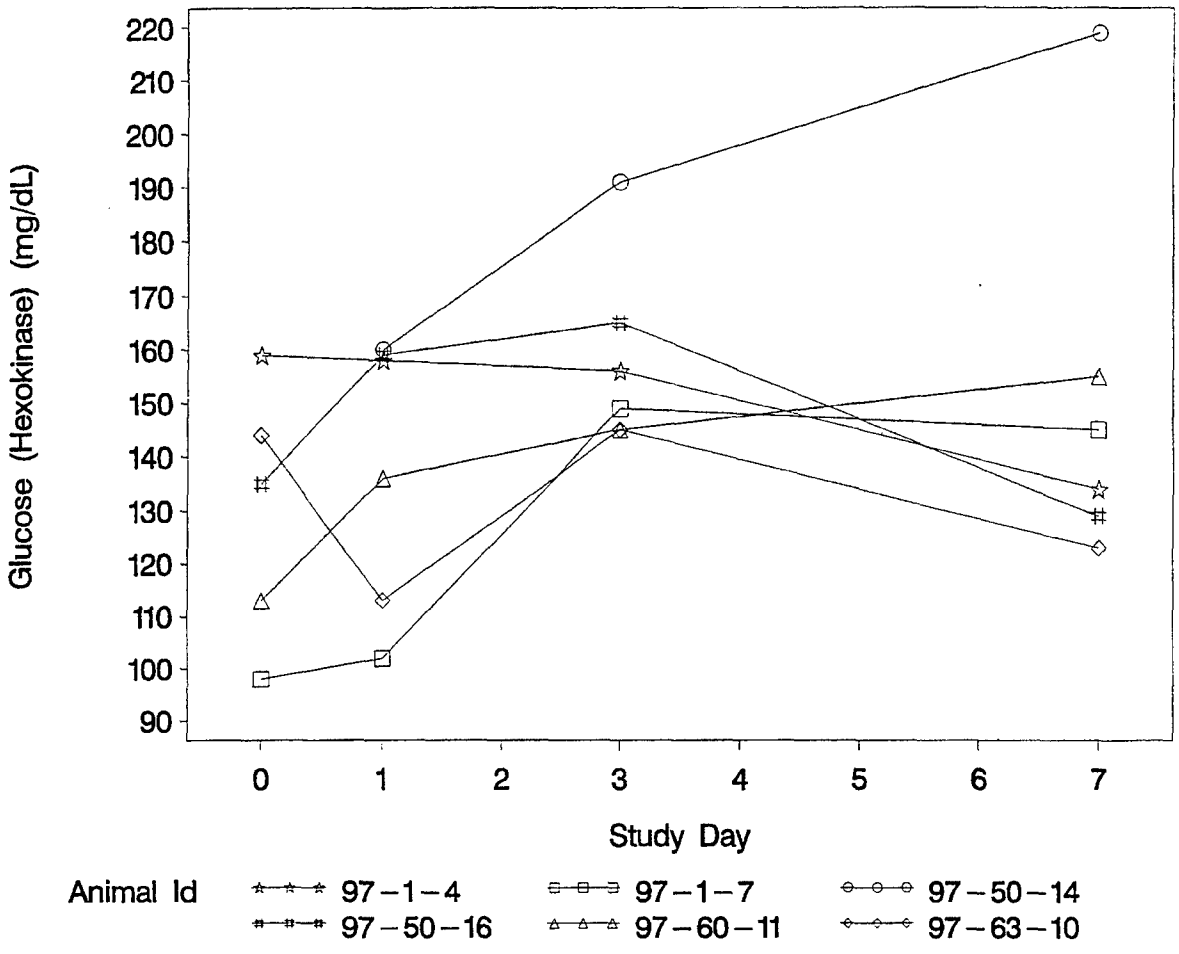

Figure B-15. Glucose (Hexokinase) (mg/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

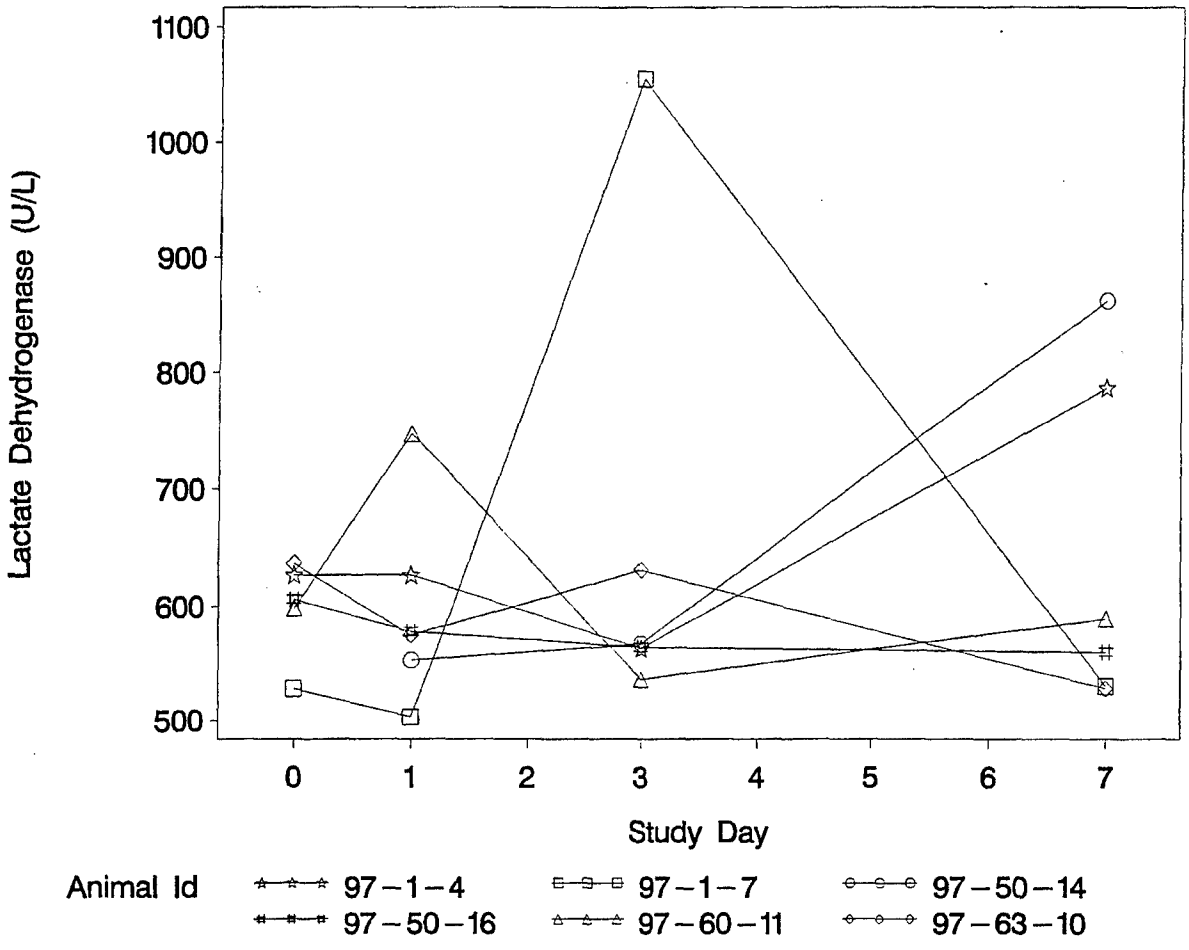

Figure B-16. Lactate Dehydrogenase (U/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

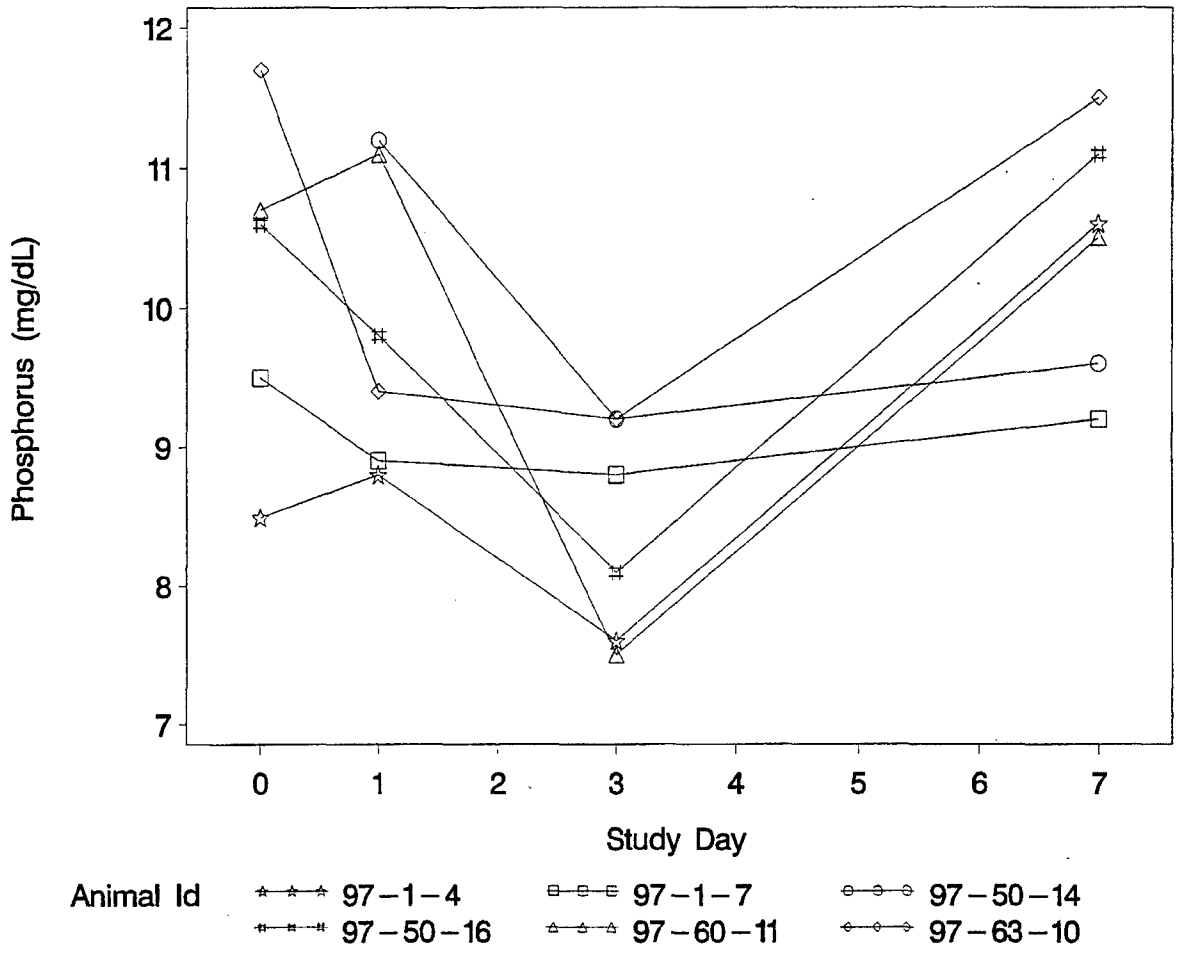

Figure B-17. Phosphorus (mg/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

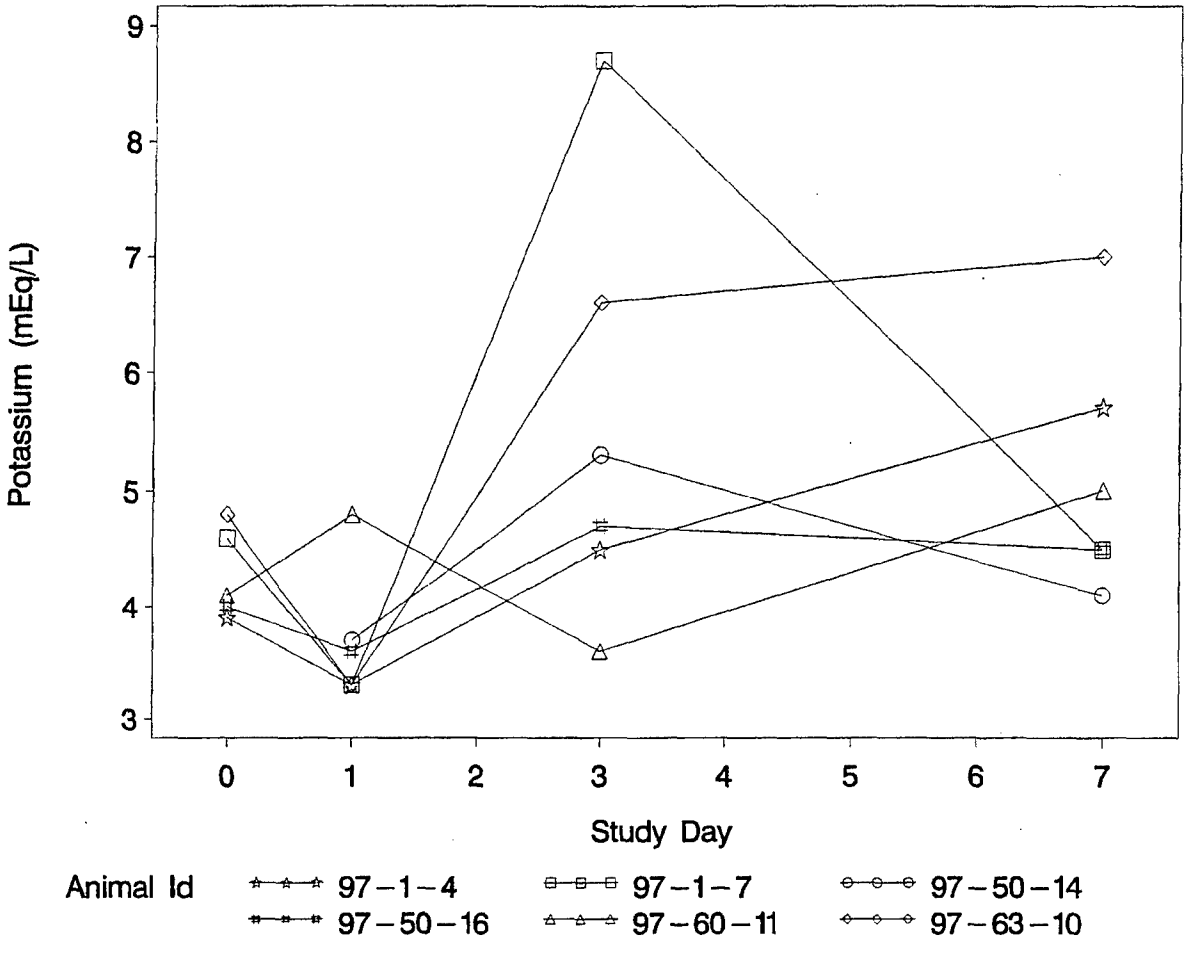

Figure B-18. Potassium (mEq/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

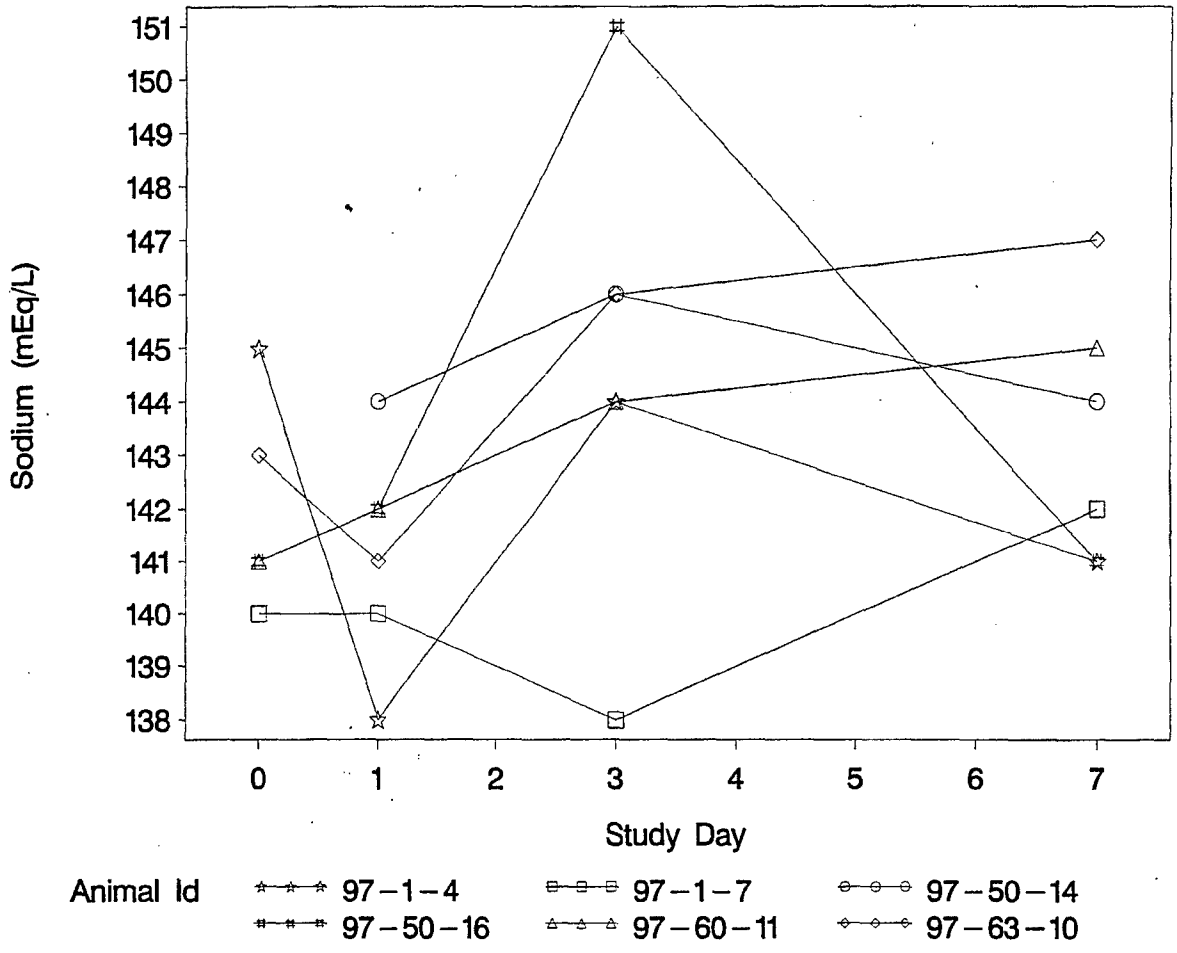

Figure B-19. Sodium (mEq/L) by Study Day of Six Different Animals Tested in Phase 1, Part B.

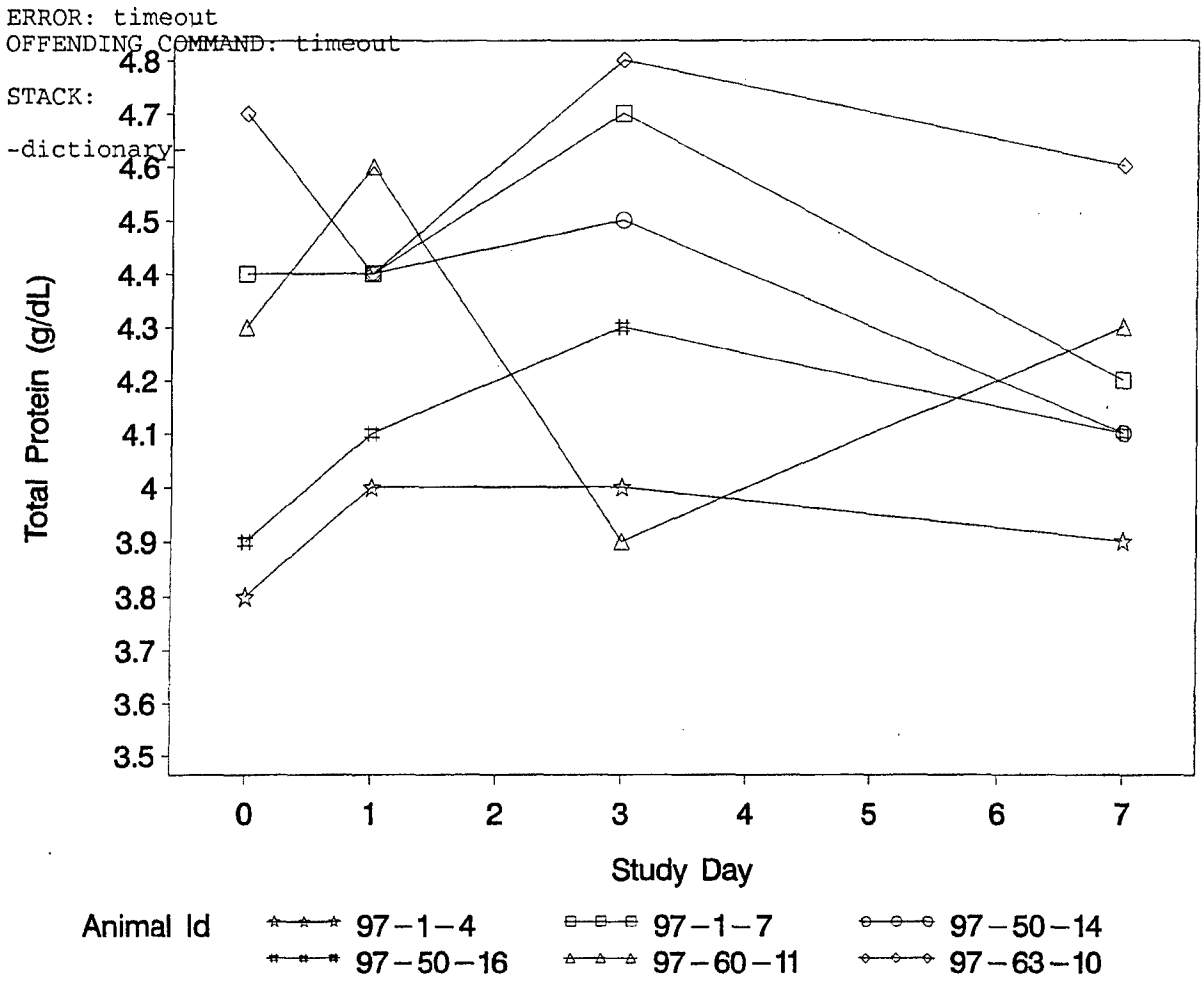

Figure B-20. Total Protein (g/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

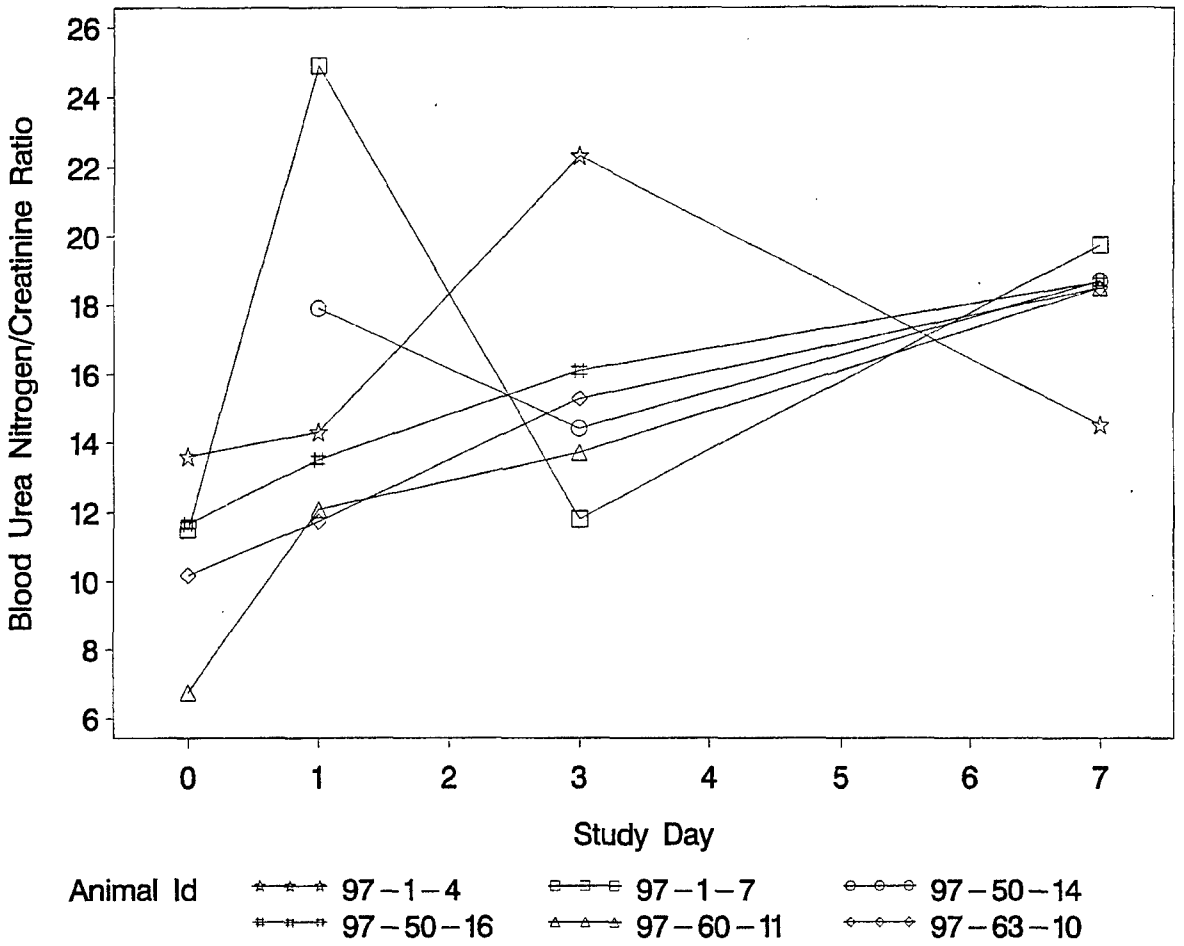

Figure B-21. Ratio of Blood Urea Nitrogen to Creatinine by Study Day of Six Different Animals Tested in Phase 1, Part B.

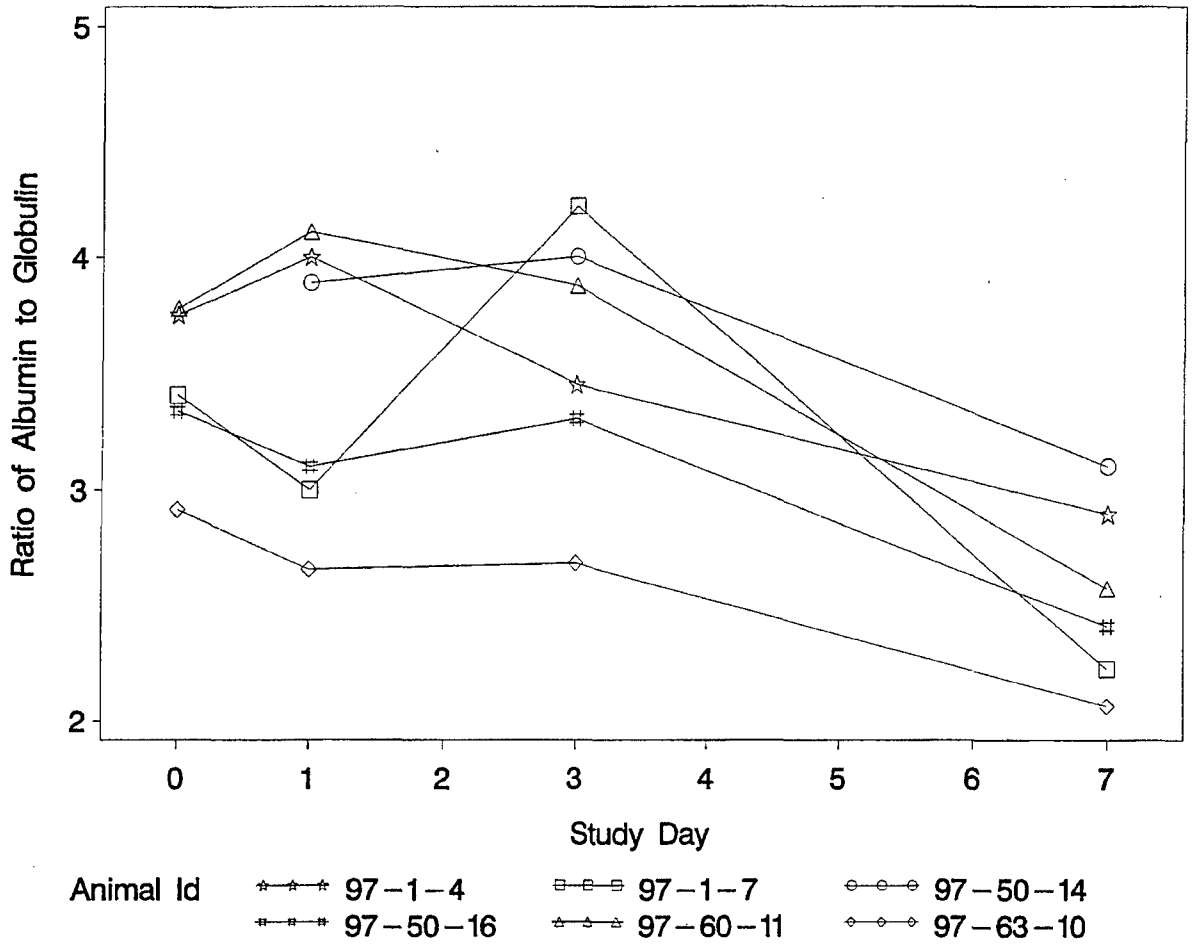

Figure B-22. Albumin to Globulin Ratio by Study Day of Six Different Animals Tested in Phase 1, Part B.

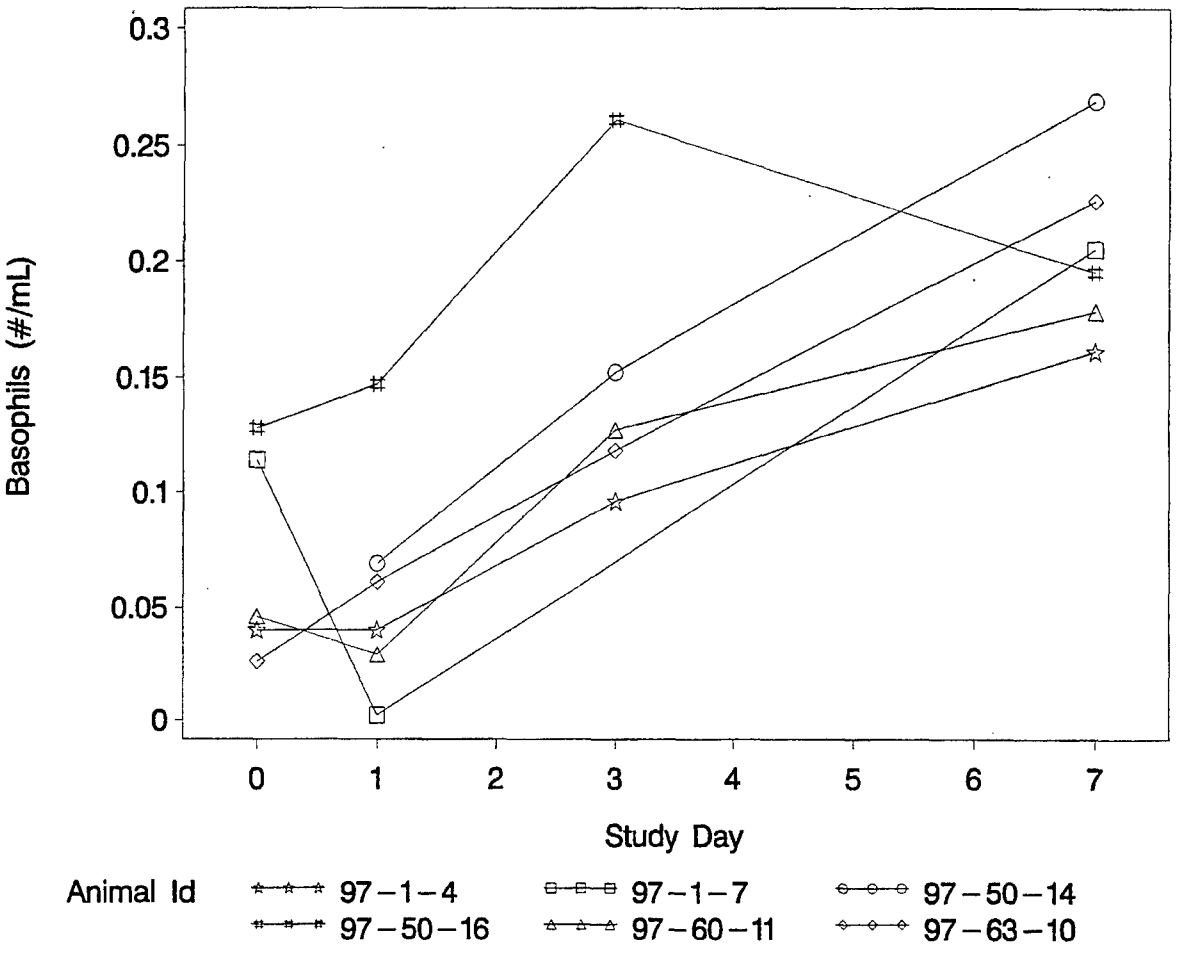

Figure B-23. Basophils (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

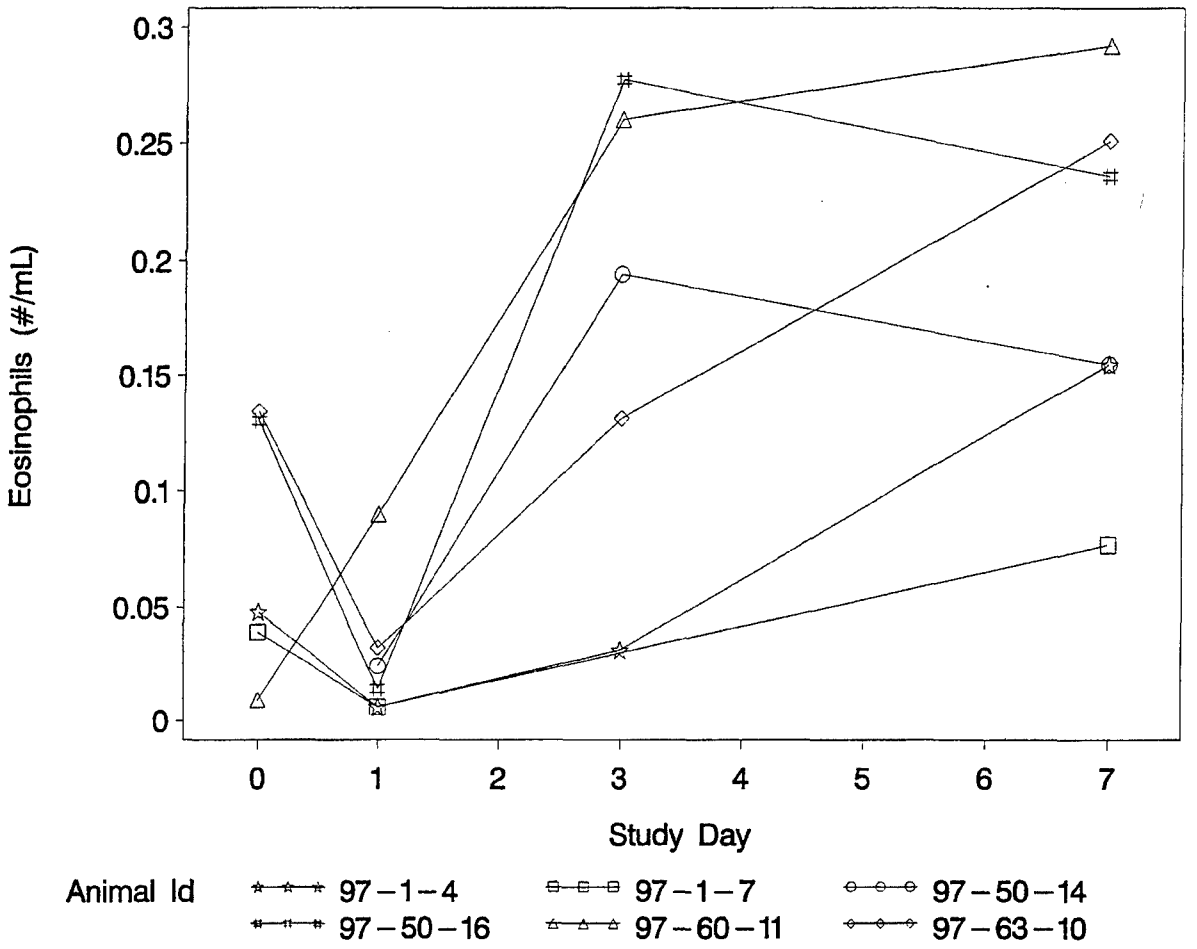

Figure B-24. Eosinophils (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

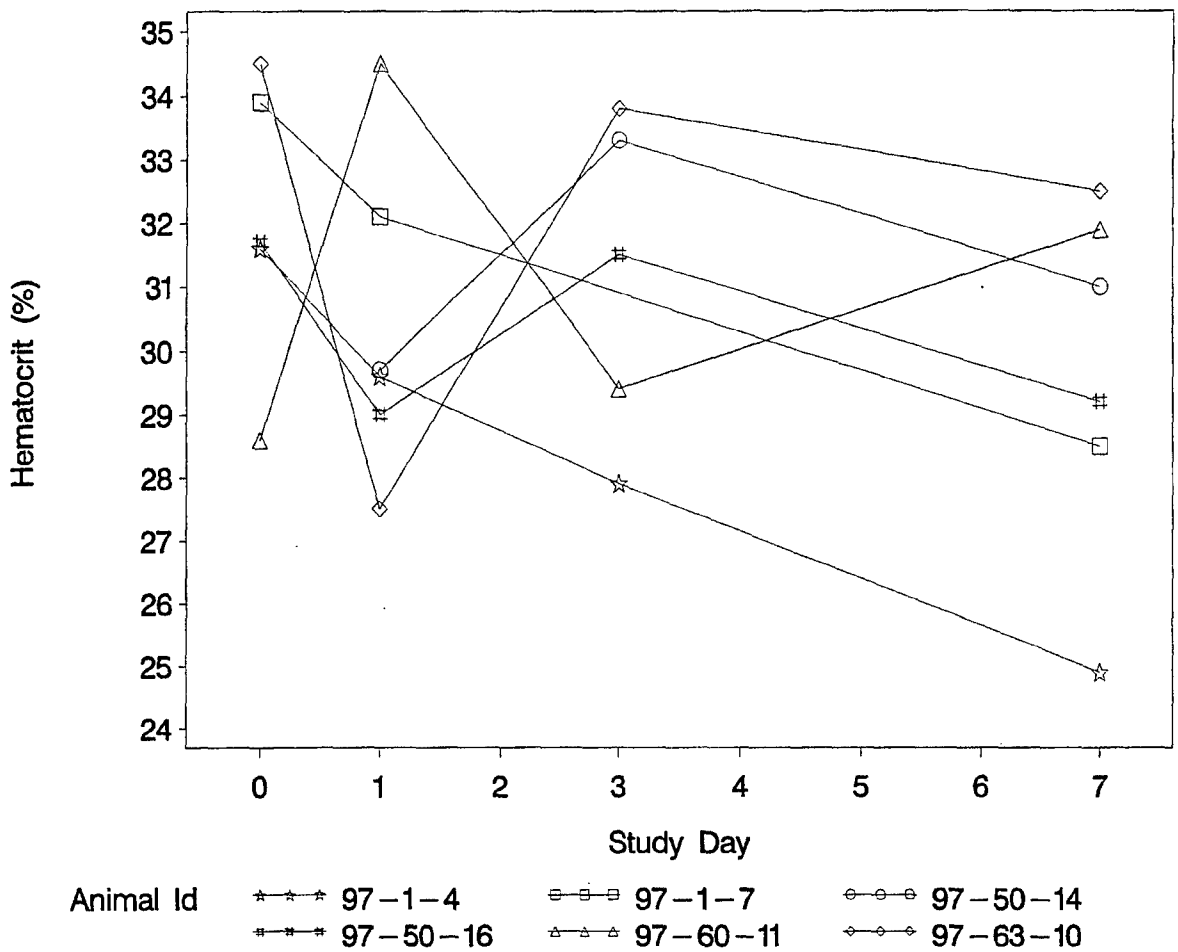

Figure B-25. Hematocrit (%) by Study Day of Six Different Animals Tested in Phase 1, Part B.

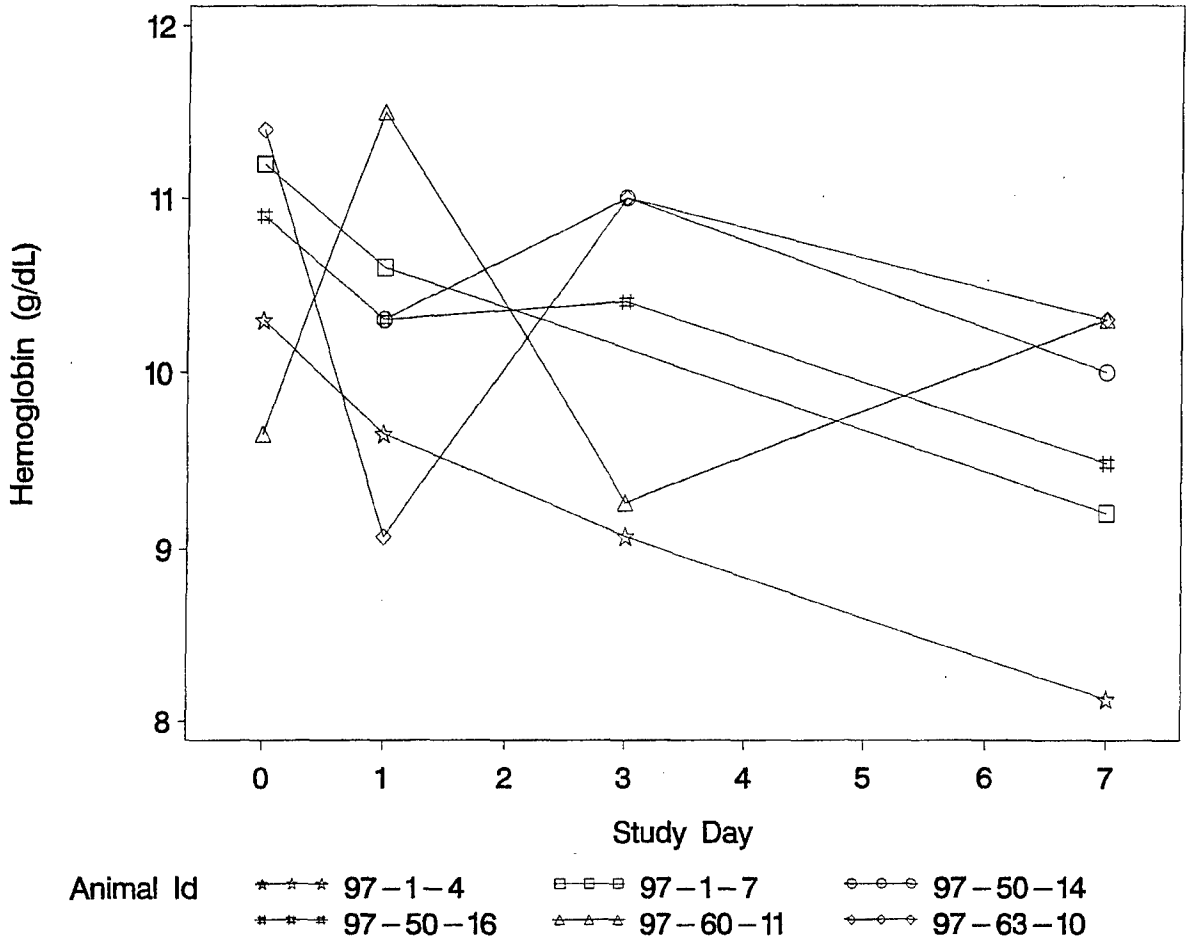

Figure B-26. Hemoglobin (g/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

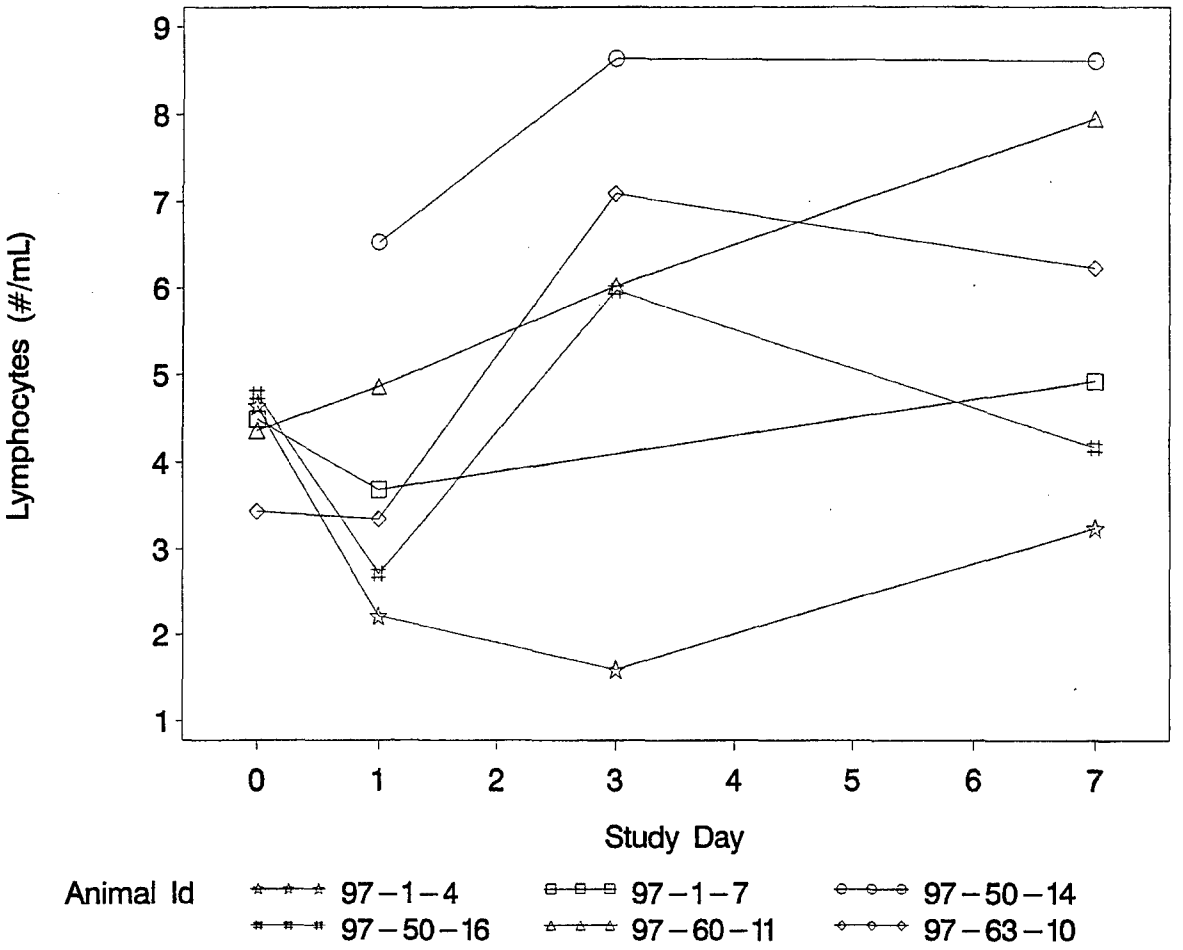

Figure B-27. Lymphocytes (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

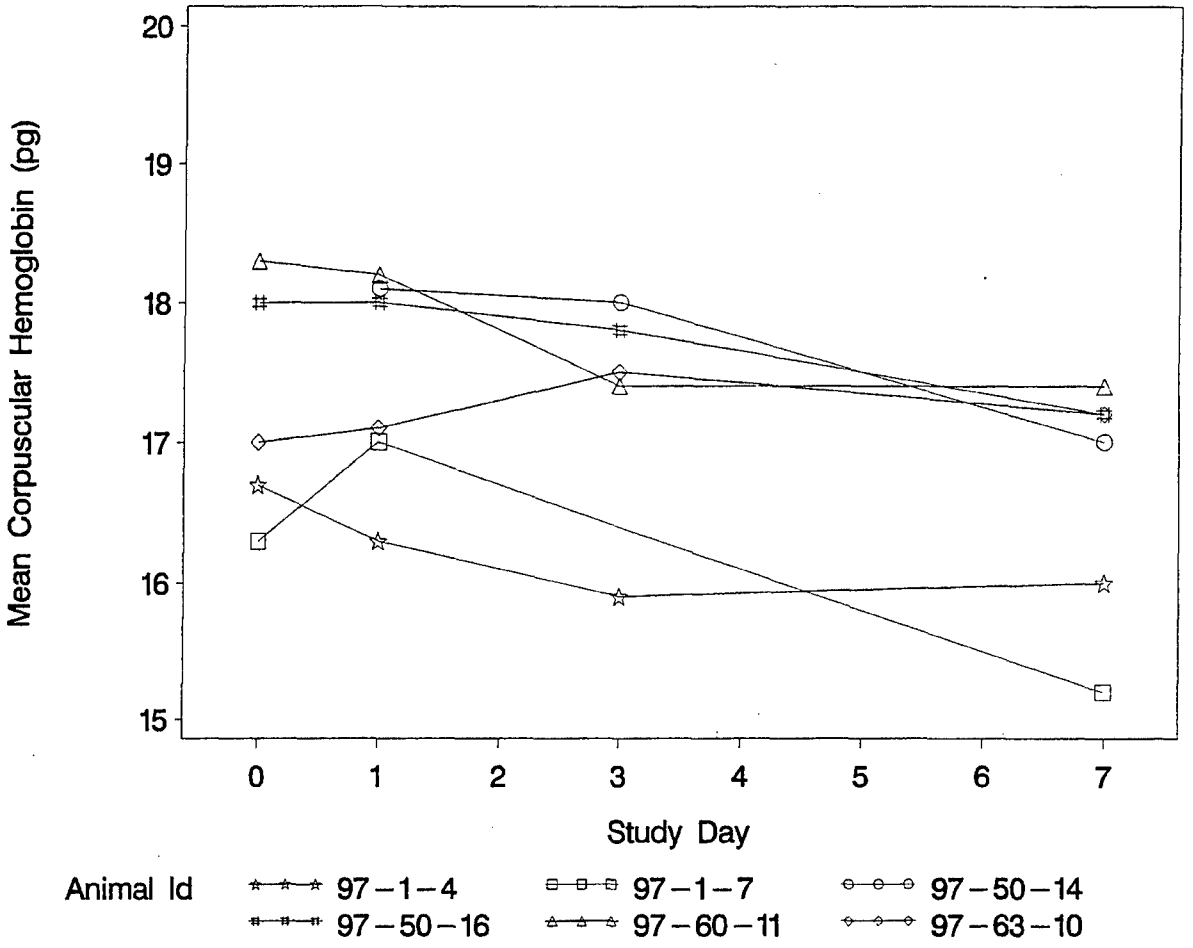

Figure B-28. Mean Corpuscular Hemoglobin (pg) by Study Day of Six Different Animals Tested in Phase 1, Part B.

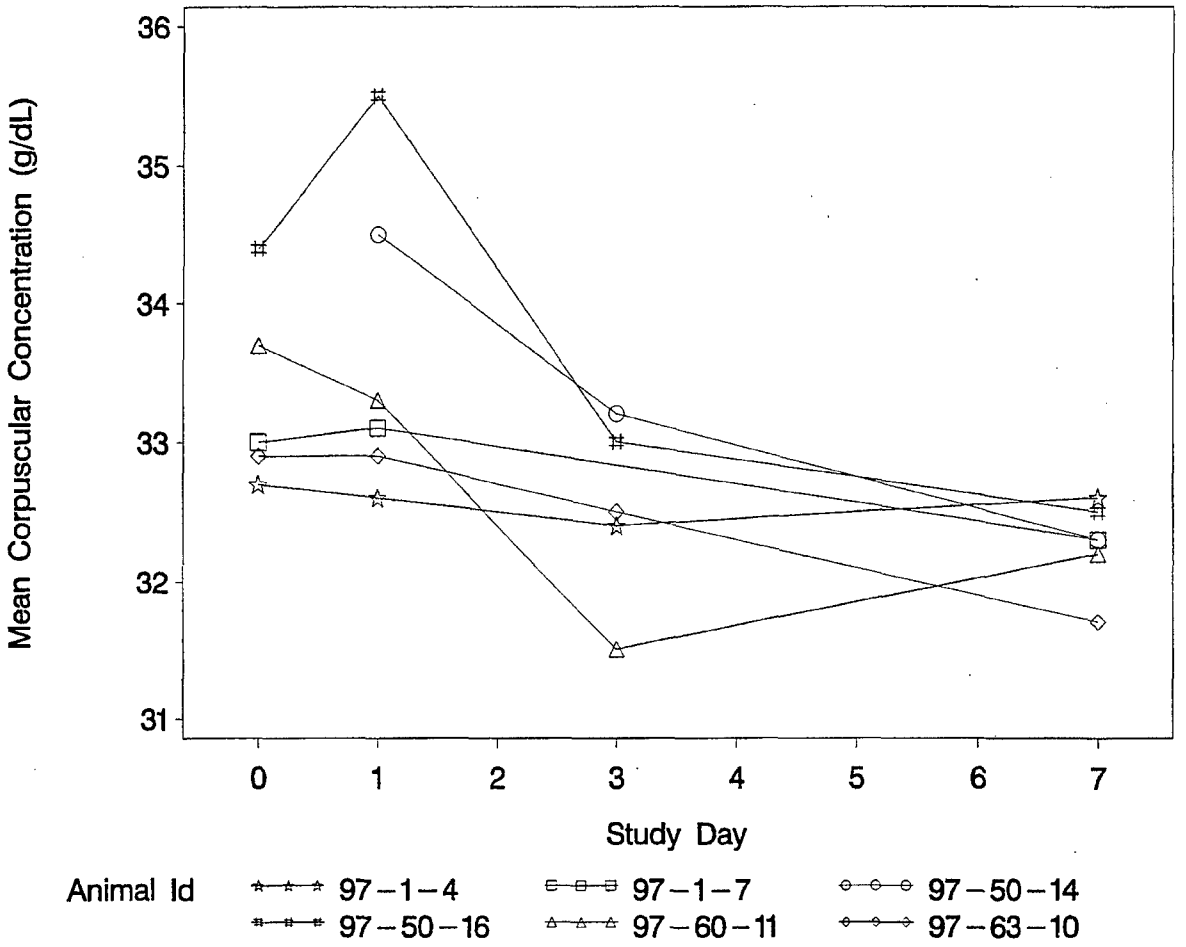

Figure B-29. Mean Corpuscular Concentration (g/dL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

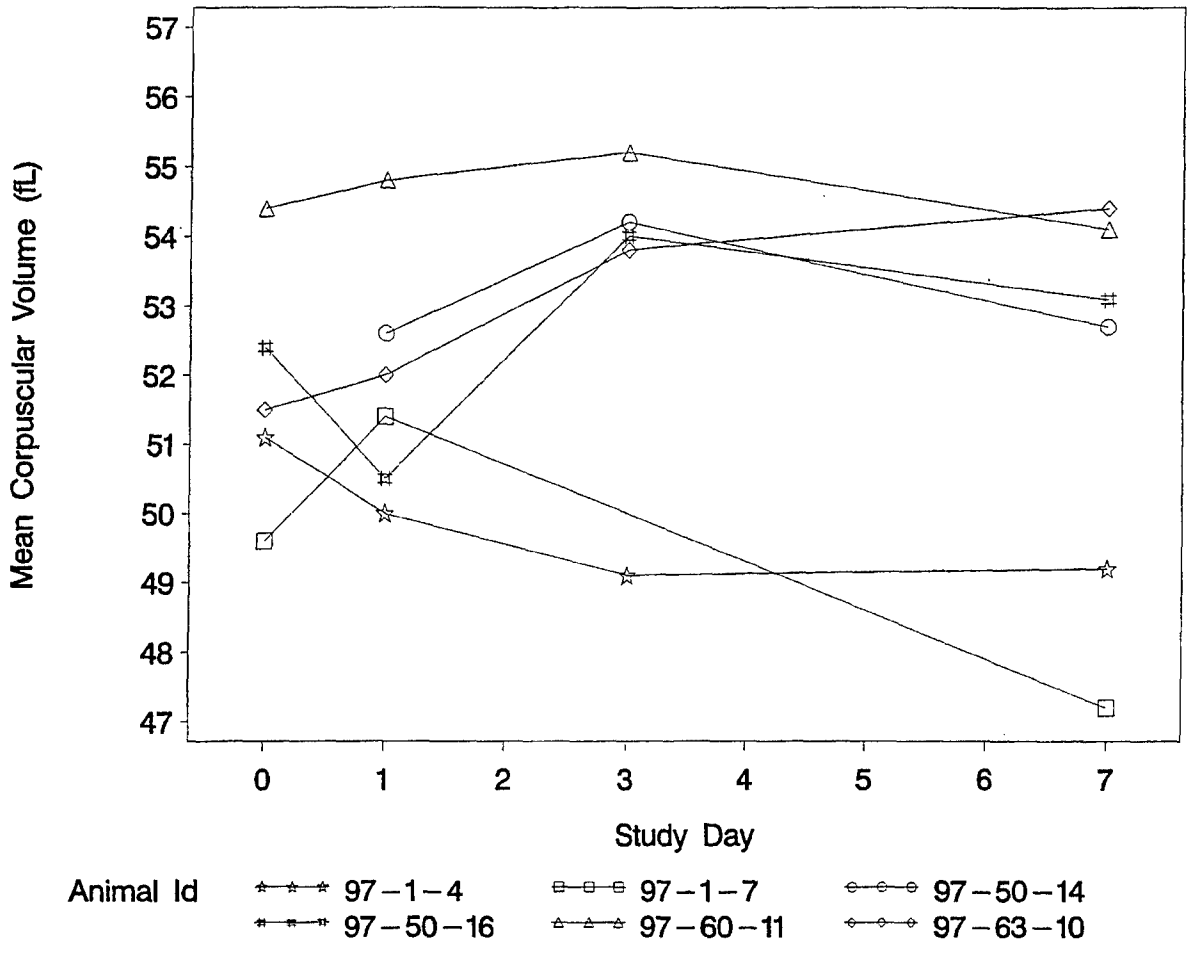

Figure B-30. Mean Corpuscular Volume (fL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

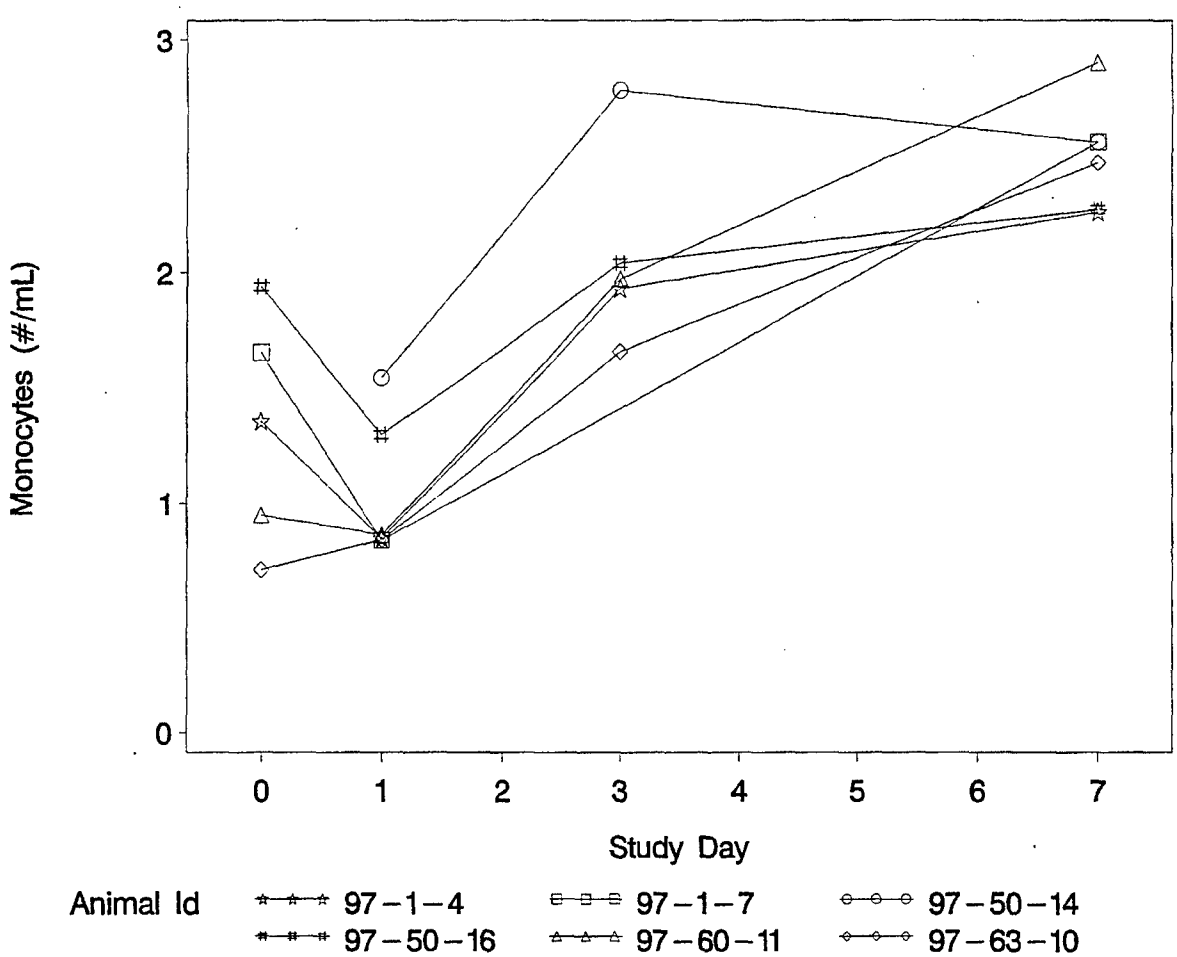

Figure B-31. Monocytes (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

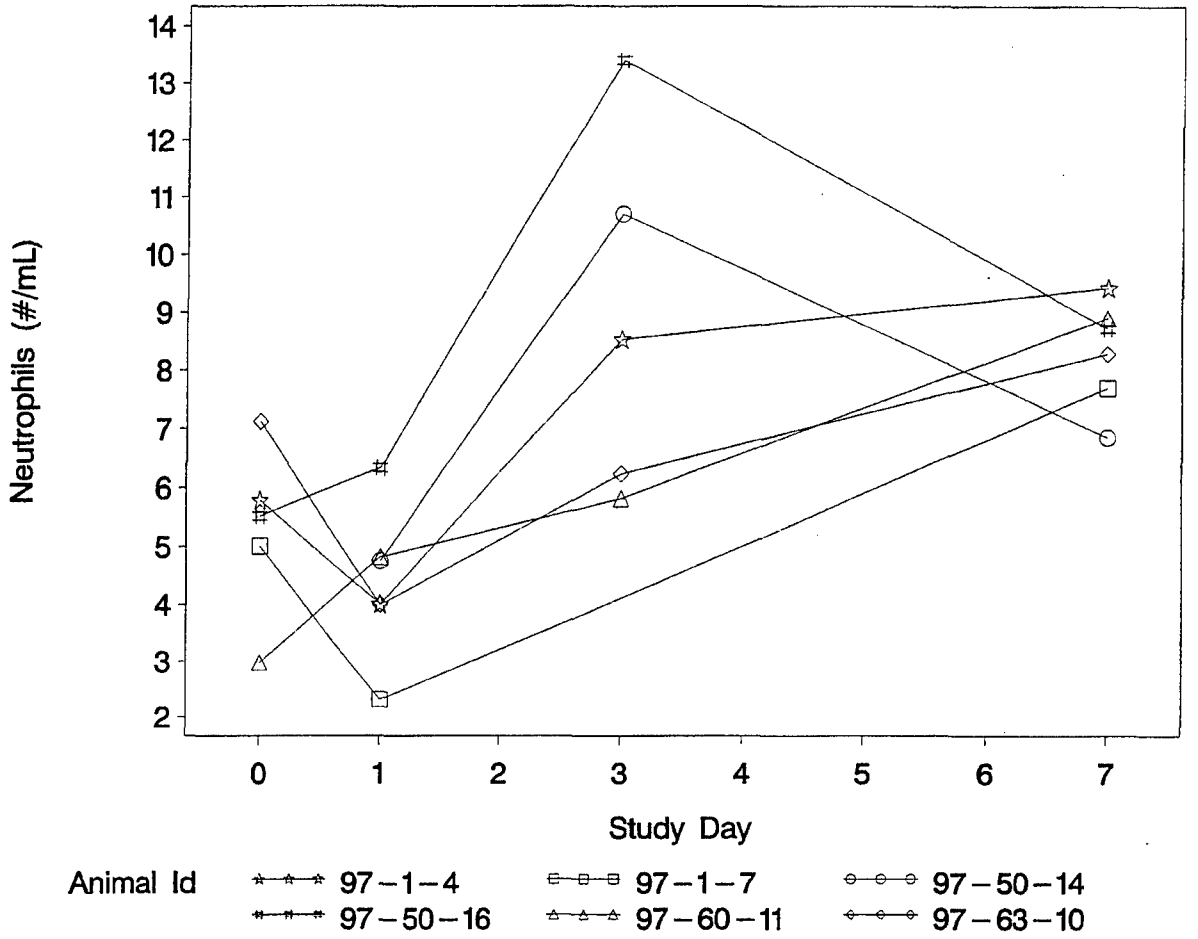

Figure B-32. Neutrophils (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

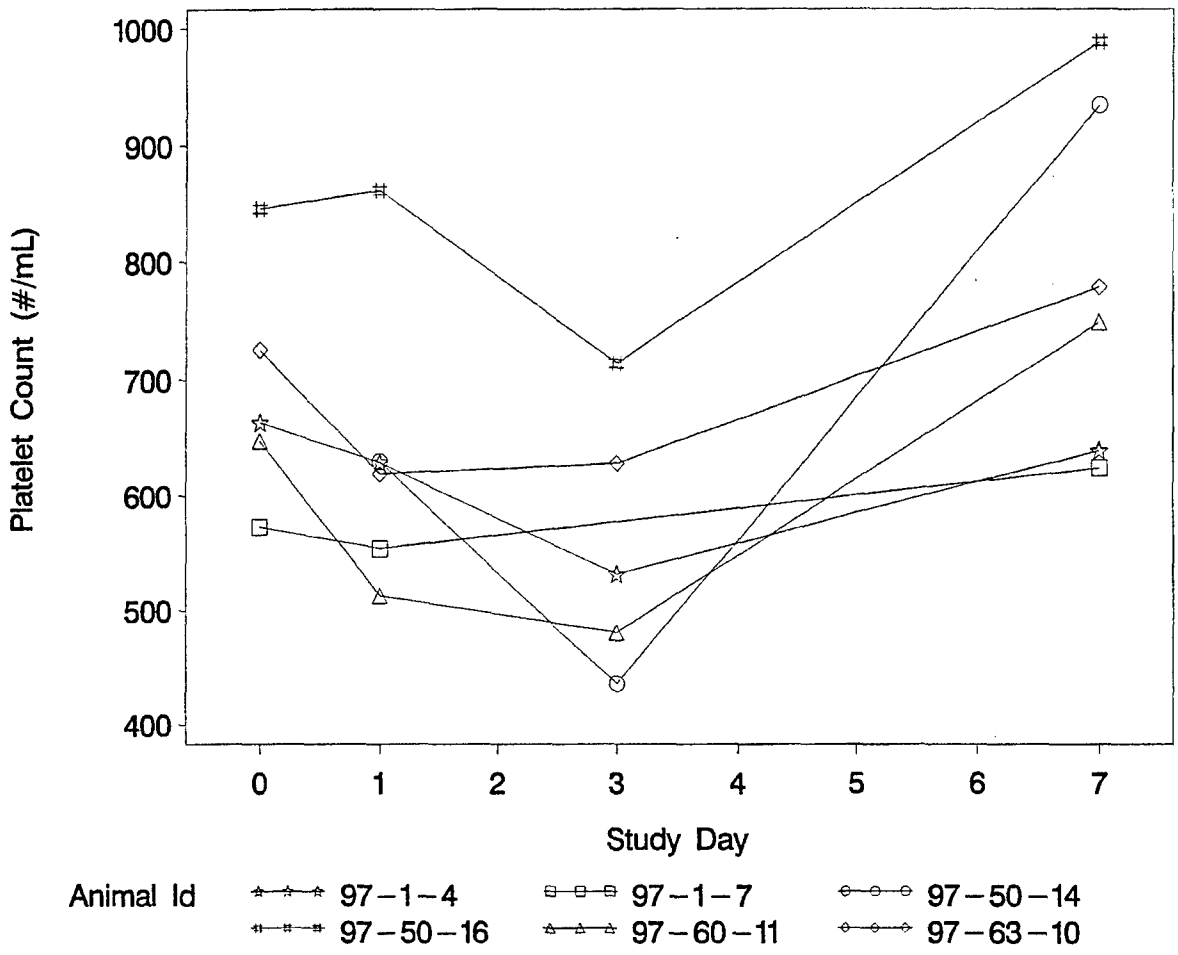

Figure B-33. Platelet Count (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

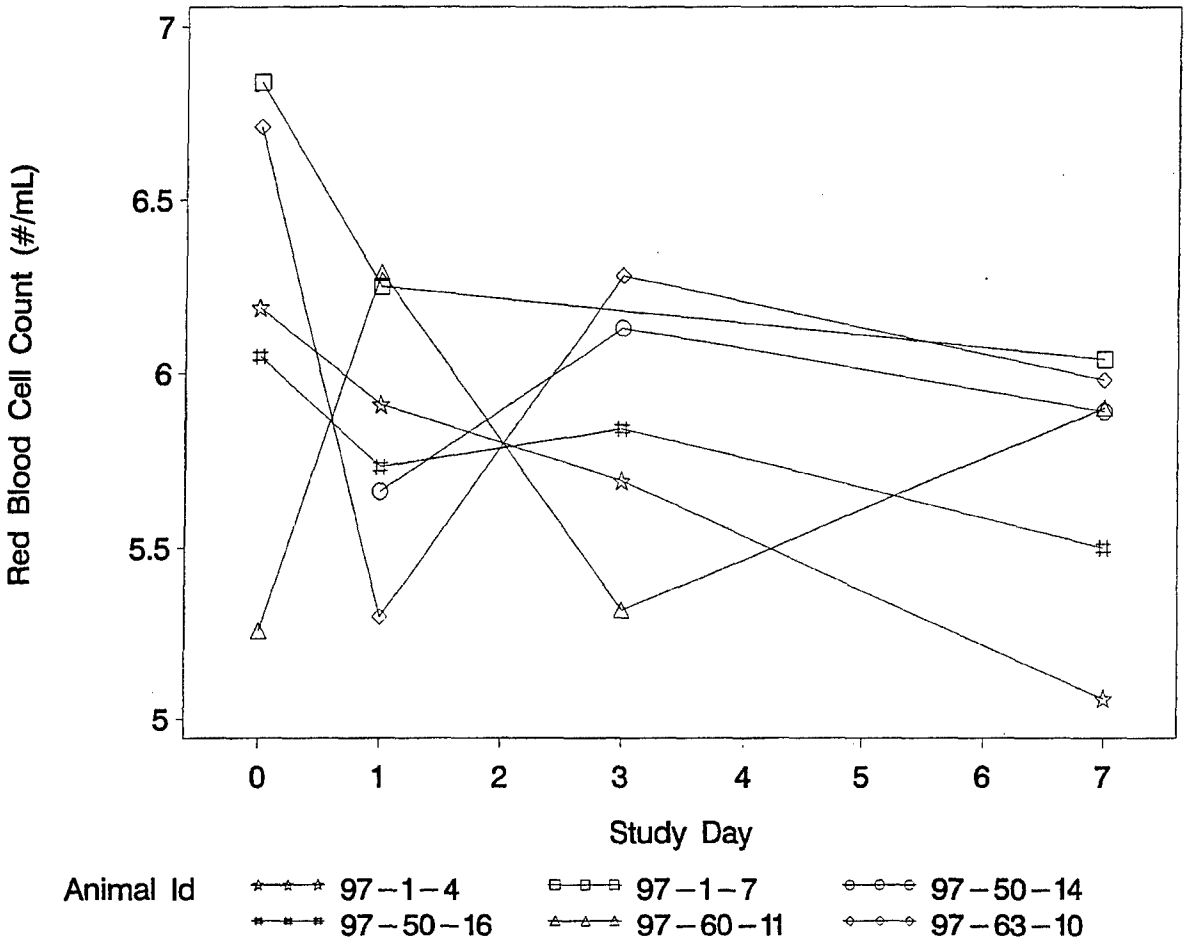

Figure B-34. Red Blood Cell Count (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

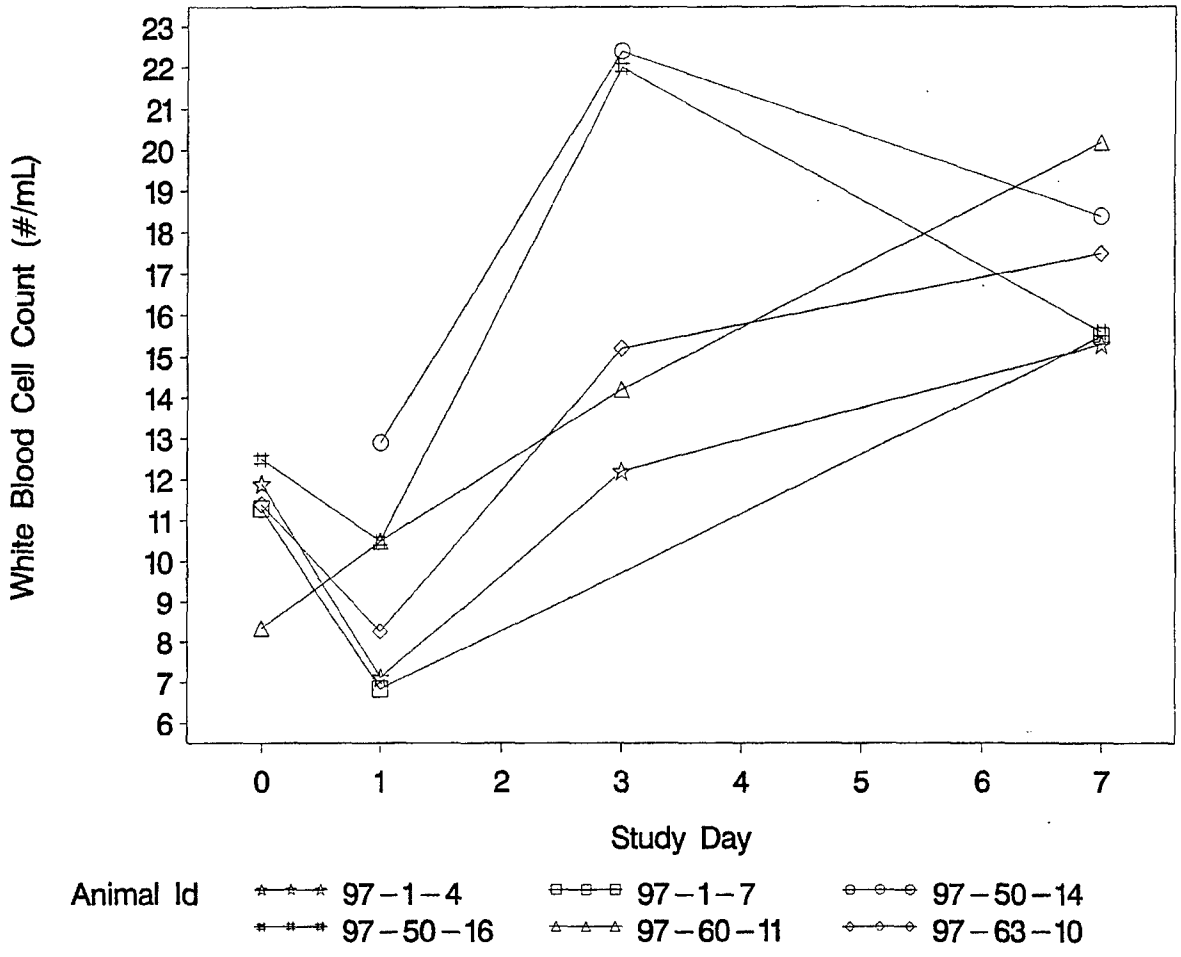

Figure B-35. White Blood Cell Count (#/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

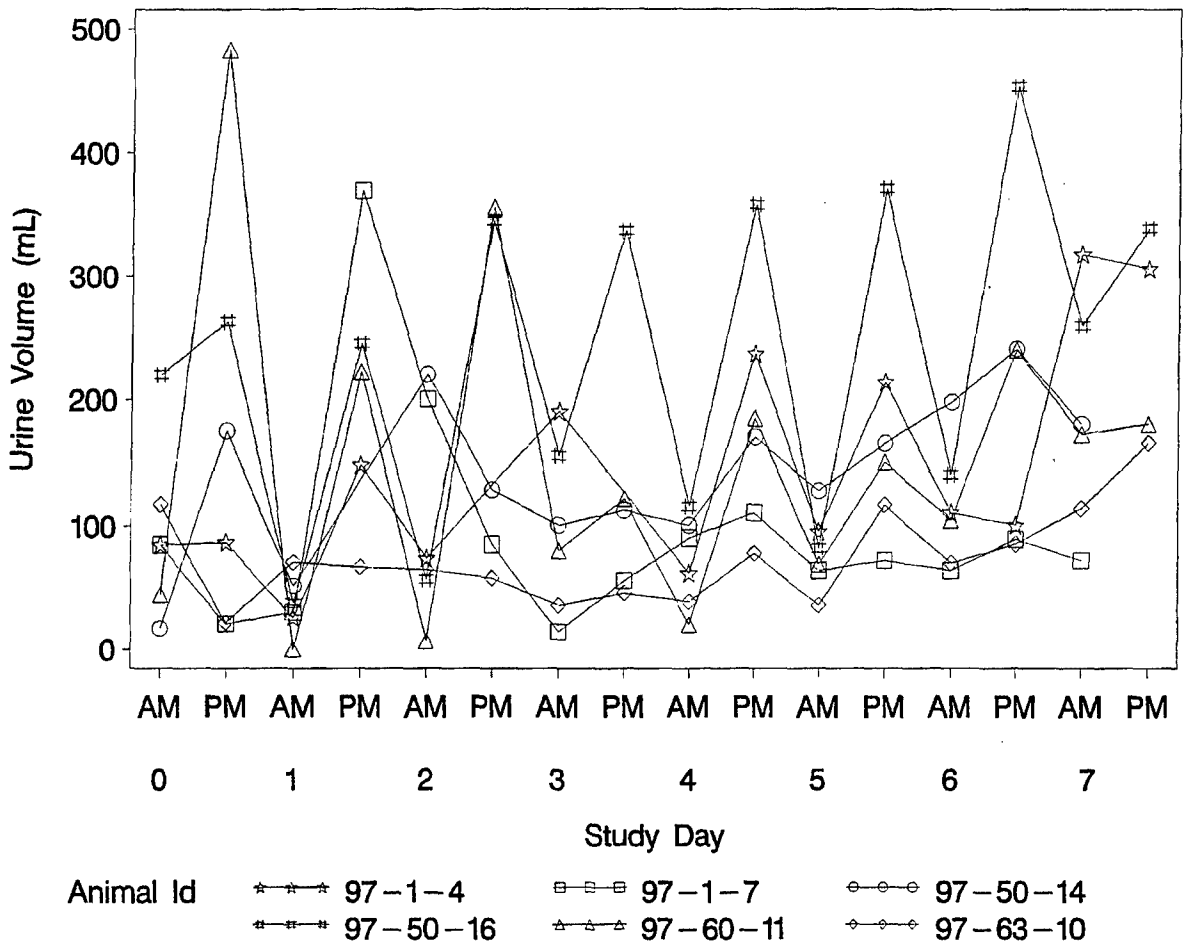

Figure B-36. Urine Volume (mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

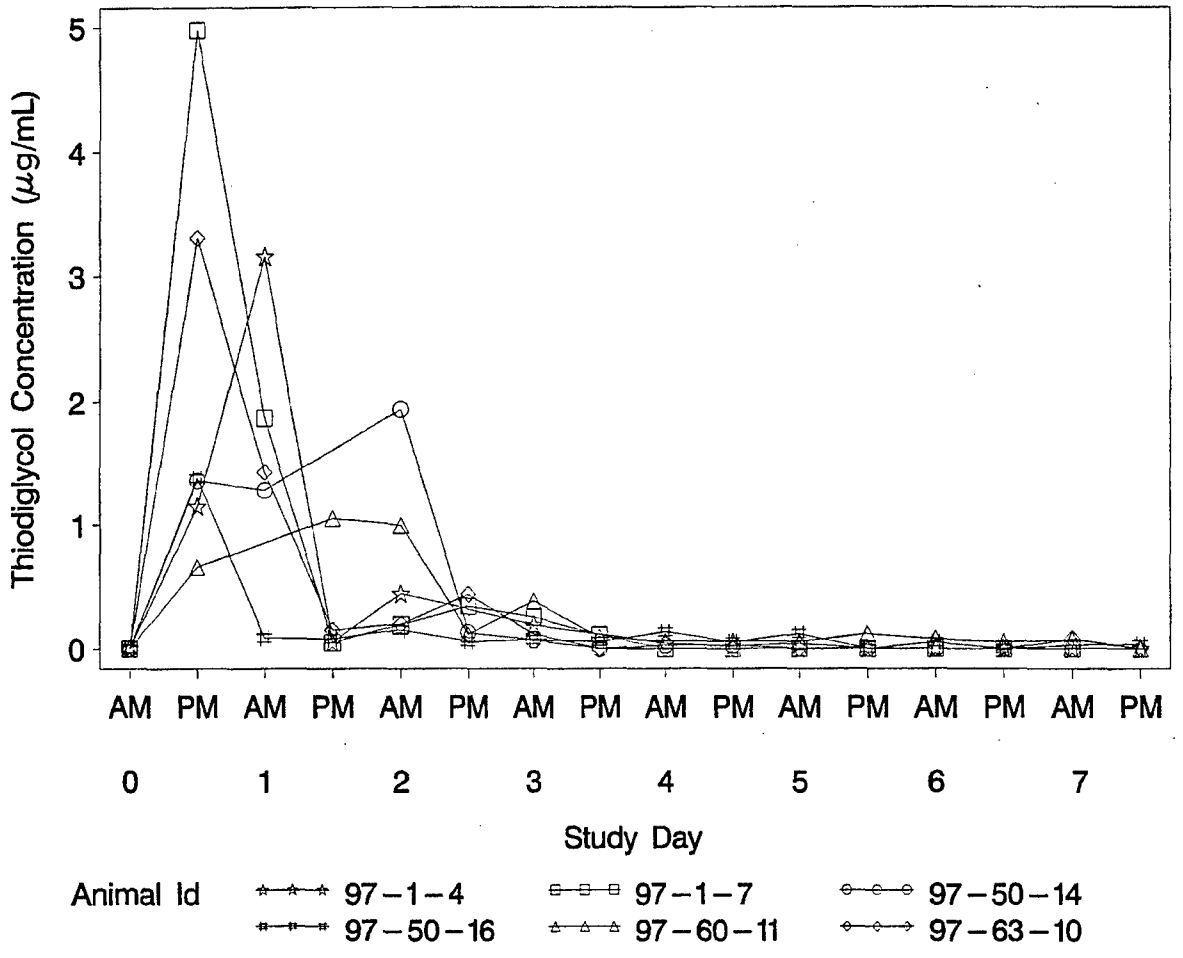

Figure B-37. Thiodiglycol Concentration (µg/mL) by Study Day of Six Different Animals Tested in Phase 1, Part B.

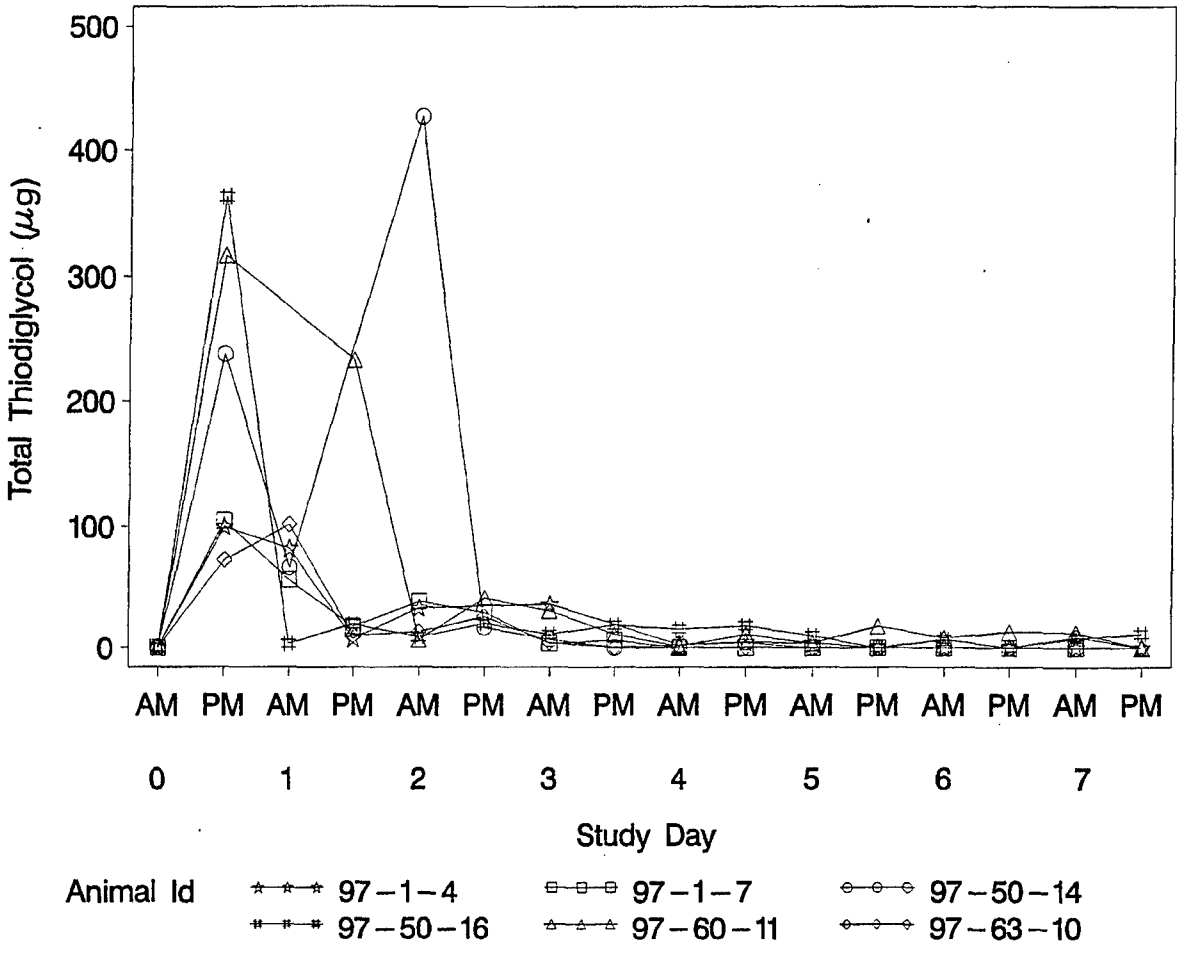

Figure B-38. Total Thiodiglycol (µg) by Study Day of Six Different Animals Tested in Phase 1, Part B.

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B. Skarpness
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Date July 16, 1998

To Frances Reid

From Shawn M. Shumaker

s:\niem\mref\task33\phaseIB letter report revised July 14.wpd

Subject **Phase I, Part B of Task 94-33 (Revised)**

Attached is the revised statistical report on physiological and histopathological data collected in MREF Task 94-33, Phase I, Part B. A WordPerfect 8 file with the text, tables, and figures will be sent via e-mail for inclusion into your draft report.

This report updates the report dated February 5, 1998 as follows:

1) ANOVA models were fitted using Proc Mixed, not Proc GLM as indicated in the previous report.
2) Since clinical observations were taken prior to wound debridement, the debriding effect was removed from the ANOVA.
3) A likelihood ratio test was used to determine whether animal-to-animal variability was significant.
4) Table 1 was modified to present results as they were presented for the SOT poster presentation in March.

No change was made to the summary of serum chemistry, hematology, and urinalysis parameters in this report. Additional analysis of these parameters was provided in the April 16, 1998 memo, "Additional Analysis of Task 33, Phase IB Data."

SMS:mk

Attachment

For Review and Approval

	Name	Initials	Date
Originator	S. Shumaker	SMS	7/16/98
Concurrence	N. Niemuth	N	7/16.98
Approved	B. Skarpness	BS	7/16/98

STATISTICAL REPORT FOR MREF TASK 94-33, PHASE I, Part B (6 ANIMALS)

BACKGROUND

This report summarizes data collected on the 6 animals in Phase I, Part B of Task 94-33. These 6 animals were used to evaluate specific physiological, histopathological, and clinical observation endpoints over a seven day period. Urinalysis, hematological cell counts, and specified serum chemistry samples were collected and measured twice a day through 7 days on the study. Histopathological endpoints were evaluated on day 2 and day 7. Clinical observations were taken on day 2.

STATISTICAL SUMMARY

Descriptive and/or summary statistics are presented for histopathology, clinical observations, serum chemistry, hematology, and urinalysis of Phase I, Part B animals. Unless otherwise noted no statistical evaluations of values nor their trends over time were conducted. However, upon visual inspection of the data, possible trends were noted. These observations were based only upon inspection of the data by the statistician and are made with little or no knowledge of the endpoints and their normal values or ranges.

HISTOPATHOLOGY

The mean and percent incidence of histopathological endpoints are summarized in Table 1. Necrosis was present in all samples taken from the center of the exposed site on day 2 and day 7 regardless of debriding and was present in all but one peripheral sample on day 2. The one sample without sign of necrosis was a debrided site. The mean grade for depth of necrosis was near maximum (4) at 3.9 for both debrided and not debrided center sites on day 2. Depth of necrosis was graded at the maximum score of 4 for all center sites on day 7. The mean grade for depth of necrosis on day 2 was lower for peripheral sites than center sites, 3.6 for debrided and 3.8 for not debrided. Necrosis of the basal epithelium was present and at maximum grade (4) in all samples on day 2 regardless of debriding or sample location. At day 7 necrosis of the basal epithelium was still present at maximum grade (4) in all samples. Ulceration was present in only one sample on day 2, a peripheral debrided site, but was present in all samples on day 7. There was no sign of tissue granulation in day 2 samples, but on day 7 tissue granulation was present in all samples. The mean tissue granulation grade on day 7 was 2.8 for debrided sites and was 2.4 for sites not debrided. No reepithelialization occured by day 7.

CLINICAL OBSERVATIONS

Descriptive statistics for clinical observations are presented in Table 2. Exudate, erythema, edema, necrosis, and eschar were evaluated on day 2. Eschar was not present on these days and was excluded from the table. Wound size (WS) was measured for each site.

Erythema, necrosis, and edema were present in all sites on study day 2. Erythema and edema scores tended to be the most severe with average scores of 4.68 and 4.47 respectively while necrosis was more moderate with an average score of 1.23. Exudate was observed infrequently, except on animal 97-63-10, where it was observed in 5 of 6 sites. When observed, exudate was mild, except in the anterior sites of animal 97-63-10 where the values were 3 and 2.25. Average WS was 1,565 mm^2. The mean values of exudate, erythema, edema, necrosis, and WS for each animal, overlaid on the observed values for each site are presented in Figures 1-5 respectively.

Analysis of variance (ANOVA) models were fit to the exudate, erythema, edema, necrosis, and WS data to assess the animal-to-animal variability and to determine if there was a significant difference among sites. Appropriate contrasts were also used to assess whether there were differences between anterior and posterior sites. The ANOVA model took the following form:

$$Y_{ij} = \mu+\alpha_i+\gamma_j+\epsilon_{ij}$$

where Y_{ij} is a clinical observation, i indicating site, and j indicating the animal; μ is the average; α_i is the fixed site effect; γ_j is a random animal effect; and ϵ_{ij} is a random error term. The SAS (ver. 6.12) Mixed procedure was used to fit the ANOVA models. A likelihood ratio test was used to determine whether animal-to-animal variability was significant. Results are presented in Table 3.

The overall site effect was statistically significant for WS, exudate, and edema. WS scores were significantly greater on posterior sites than anterior sites as were edema scores. Exudate scores were significantly smaller on posterior sites than anterior sites. Animal-to-animal variability was significant for Exudate, Erythema, Edema, and WS. No significant effects were detected in the analysis of necrosis.

SERUM CHEMISTRY

Descriptive statistics for serum chemistry values for the pre-study physical through day 7 are presented in Table 4. The mean, minimum, and maximum values per day are also presented in Figures 6 through 24. No statistical evaluations of these values or their trends over time were conducted. However, upon visual inspection of the data a few possible trends were noted.

Figure 8 shows that alkaline phosphatase declines from a mean of 450.5 U/L during the physical to a mean of 343.4 U/L on the day of dosing. It continues to decline after dosing to a mean of 252.5 U/L on day 7. Figure 11 shows mean blood urea nitrogen levels rising from

10.0 mg/dL on day 0 to a approximately 16 mg/dL on days 1, 3, and 7. The ratio of blood urea nitrogen to creatinine profile (Figure 23) is similar to the blood urea nitrogen profile as creatinine levels do not change greatly through most of the study. However, as seen in Figure 15, creatinine levels are somewhat higher on day 1 and day 3.

HEMATOLOGY

Descriptive statistics for hematolgy data for the pre-study physical through day 7 are presented in Table 5. The mean, minimum, and maximum values per day are also presented in Figures 25 through 37. No statistical evaluations of these values nor their trends over time were conducted. Upon visual inspection of the data one notices trends in almost every endpoint to one degree or another. Those hematology values with the most visually apparent trends are pointed out in the following paragraph.

Between day 0 and day 7 mean basophil counts (Figure 25) rise from 0.071 #/mL to 0.206 #/mL. Mean corpuscular concentration (Figure 31) decreases over the course of the study from a mean of 33.340 g/dL on day 0 to 32.267 g/dL on day 7. Mean monocyte levels (Figure 33) rise from 1.324 #/mL to 2.503 #/mL between day 0 and day 7. Mean neutrophil (Figure 34) values rise from 5.284 #/ml on day 0 to 8.320 #/ml on day. White blood cell counts (Figure 37) rise from a mean of 11.090 #/ml on day 0 to a mean of 17.083 on day 7. Mean neutrophil and white blood cell counts were similar on days 3 and 7, however, greater variability was present on day 3.

URINALYSIS

Descriptive statistics for urine volume and thiodiglycol concentrations for the morning (AM) of day 0 through the evening (PM) of day 7 are presented in Table 6. The mean, minimum, and maximum values are also presented in Figures 38 and 39. The distributions of the results from the reagent strips for urinalysis are presented in Table 7. No statistical evaluations of these values nor their trends over time were conducted. However, upon visual inspection of the data a few possible trends were noted.

Urine volume increased slightly toward the end of the study. Mean thiodiglycol values were 0 on day 0 (AM). Twelve hours later, they peaked at a maximum mean value of 2.14 after which they declined to near 0 by the end of the study.

As shown in Table 7, glucose was detected in the urine of only two animals. On day 1 (PM), 100 mg/dL of glucose was detected in one animal and on day 1 (PM) and day 2 (AM), 1000 mg/dL and 2000 mg/dL respectively were detected in the other. Small amounts of bilirubin were detected in one animal on days 5 (AM) and 5 (PM). Trace amounts of ketone (5 mg/dL) were detected in one animal on day 3 (AM) and then again in another animal on day 7 (AM). Blood levels in urine rose through day 3 (PM) where 83 percent of animals had large amounts of blood detected and the remaining 17 percent had moderate amounts of blood detected, and then began to decline toward the end of the study. By day 3 (AM), 67 percent of animals had 30 mg/dL protein in the urine, while the remaining 33 percent all showed trace amounts of protein.

Protein levels remained elevated at the end of the study. The test for nitrate was negative for 83 percent of animals at day 0 (AM), but was positive for all animals by day 7 (PM). Leukocytes were not detected in urine throughout the study. The pH of the urine samples was highly variable on a daily basis, but may show a decline in the first 24 hours to 48 hours followed by a gradual return to normal. Specific gravity readings were also highly variable, but appear to have increased following dosing.

Table 1. Mean and Percent Incidence of Histopathologic Wound Development and Wound Healing Endpoints for the Six Animals Tested in Phase I, Part B.

	Histopathology Endpoint	Debride Site	Center						Peripheral		
			Day 2			Day 7			Day 2		
			N	% Incidence	Mean*	N	% Incidence	Mean*	N	% Incidence	Mean*
Wound Development	Depth of Necrosis	Yes	18	100	3.9	18	100	4.0	18	94	3.6
		No	18	100	3.9	18	100	4.0	18	100	3.8
	Necrosis of Basal Epithelium	Yes	0/18	NA	NA	9/18	100	4.0	4/18	100	4.0
		No	18	100	4.0	16/18	100	4.0	18	100	4.0
	Ulceration	Yes	0/18	NA	NA	18	100	NA	4/18	25	NA
		No	18	0	NA	18	100	NA	18	0	NA
Wound Healing	Granulation Tissue Response	Yes	18	0	NA	18	100	2.8	18	0	NA
		No	18	0	NA	18	100	2.4	18	0	NA
	Reepithelialization	Yes	18	0	NA	18	0	NA	15/18	0	NA
		No	0/18	NA	NA	18	0	NA	0/18	NA	NA

* Mean was calculated only for sites where the condition was observed.

Table 2. Descriptive Statistics of Clinical Observations parameters, on Study Day 2 for the Six Animals Tested in Phase I, Part B.

Clinical Observation Parameters	N	Mean	Std. Deviation	Minimum	Maximum
Exudate	36	0.30	0.66	0.00	3.00
Erythema	36	4.68	0.37	3.75	5.50
Edema	36	4.47	0.87	2.00	5.25
Necrosis	36	1.23	0.49	0.75	3.00
WS	36	1565.17	341.18	1191.45	2591.81

Table 3. ANOVA Results for Clinical Observations for the Six Animals Tested in Phase I, Part B.

Clinical Observation Parameters	Site p-value	Animal-to-Animal Variability	Posterior-Anterior	
			p-value	Mean[± SE]
Exudate	0.0230	0.0068	0.0014	-1.42 [± 0.40]
Erythema	NS*	0.0019	NS	-0.25 [± 0.22]
Edema	0.0094	0.0389	0.0003	2.20 [± 0.53]
Necrosis	NS	NS	NS	-0.04 [± 0.36]
WS	0.0099	0.0001	0.0003	715.5 [± 172.3]

* Not significant at the 0.05 level of significance.

Table 4. Descriptive Statistics* of Serum Chemistry Parameters, by Study Day for the Six Animals Tested in Phase I, Part B.

Parameter	Study Day				
	Physical	**0**	**1**	**3**	**7**
Alanine Transaminase (U/L)	54.5 (51.0-58.0) 4.9 2	73.6 (56.0-97.0) 18.5 5	76.5 (47.0-103.0) 19.7 6	74.3 (48.0-95.0) 19.4 6	59.8 (42.0-74.0) 12.6 6
Albumin (g/dL)	3.0 (2.6-3.3) 0.5 2	3.3 (3.0-3.5) 0.2 5	3.3 (3.1-3.7) 0.2 6	3.4 (3.1-3.8) 0.3 6	3.0 (2.9-3.1) 0.1 6
Alkaline Phosphatase (U/L)	450.5 (360.0-541.0) 128.0 2	343.4 (318.0-379.0) 23.9 5	293.8 (243.0-341.0) 32.0 6	245.0 (214.0-273.0) 19.6 6	252.5 (236.0-284.0) 17.5 6
Amylase (U/L)	7365.5 (6571.0-8160.0) 1123.6 2	7753.6 (4763.0-9739.0) 1960.5 5	6299.7 (3860.0-8254.0) 1812.5 6	5880.5 (3789.0-8380.0) 1669.5 6	6467.3 (4431.0-9003.0) 1776.6 6
Aspartate Transaminase (U/L)	48.0 (44.0-52.0) 5.7 2	50.2 (42.0-56.0) 5.7 5	60.2 (40.0-79.0) 17.0 6	44.5 (25.0-103.0) 29.1 6	49.3 (40.0-72.0) 11.8 6
Blood Urea Nitrogen (mg/dL)	11.6 (11.3-11.8) 0.4 2	10.0 (5.4-13.6) 3.0 5	16.2 (12.9-22.4) 3.9 6	16.5 (13.7-20.1) 2.3 6	16.5 (14.5-18.7) 1.8 6
Calcium (mg/dL)	9.9 (9.9-9.9) 0.0 2	9.5 (9.3-9.7) 0.2 5	9.1 (8.4-9.8) 0.5 6	9.7 (8.9-10.2) 0.6 6	9.9 (9.2-10.6) 0.5 6
Chloride (mEq/L)	84.0 (79.0-89.0) 7.1 2	78.2 (72.0-88.0) 5.9 5	82.3 (77.0-88.0) 4.5 6	87.8 (77.0-97.0) 7.4 6	77.5 (73.0-82.0) 3.5 6
Creatine Phosphokinase (U/L)	675.5 (485.0-866.0) 269.4 2	636.0 (371.0-745.0) 153.0 5	915.3 (543.0-1882.0) 501.7 6	473.3 (339.0-702.0) 136.0 6	1451.0 (407.0-5262.0) 1883.3 6
Creatinine (mg/dL)	0.9 (0.9-0.9) 0.0 2	0.9 (0.8-1.1) 0.1 5	1.1 (0.9-1.2) 0.1 6	1.1 (0.9-1.5) 0.2 6	0.9 (0.8-1.0) 0.1 6

* MEAN
(MIN-MAX)
STD
N

Table 4. Descriptive Statistics* of Serum Chemistry Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
Globulin (TP-ALB) (g/dL)	1.0 (0.9-1.1) 0.1 2	1.0 (0.8-1.2) 0.2 5	1.0 (0.8-1.2) 0.1 6	1.0 (0.8-1.3) 0.2 6	1.2 (1.0-1.5) 0.2 6
Glucose (Hexokinase) (mg/dL)	158.0 (153.0-163.0) 7.1 2	129.8 (98.0-159.0) 24.4 5	138.0 (102.0-160.0) 25.5 6	158.5 (145.0-191.0) 17.7 6	150.8 (123.0-219.0) 35.3 6
Lactate Dehydrogenase (U/L)	654.5 (635.0-674.0) 27.6 2	599.2 (528.0-637.0) 42.8 5	597.2 (503.0-747.0) 83.7 6	652.7 (536.0-1055.0) 199.6 6	642.5 (528.0-862.0) 144.4 6
Phosphorus (mg/dL)	9.0 (7.7-10.3) 1.8 2	10.2 (8.5-11.7) 1.2 5	9.9 (8.8-11.2) 1.1 6	8.4 (7.5-9.2) 0.8 6	10.4 (9.2-11.5) 0.9 6
Potassium (mEq/l)	4.0 (3.8-4.2) 0.3 2	4.3 (3.9-4.8) 0.4 5	3.7 (3.3-4.8) 0.6 6	5.6 (3.6-8.7) 1.8 6	5.1 (4.1-7.0) 1.1 6
Sodium (mEq/l)	142.0 (141.0-143.0) 1.4 2	142.0 (140.0-145.0) 2.0 5	141.2 (138.0-144.0) 2.0 6	144.8 (138.0-151.0) 4.2 6	143.3 (141.0-147.0) 2.4 6
Total Protein (g/dL)	4.0 (3.5-4.4) 0.6 2	4.2 (3.8-4.7) 0.4 5	4.3 (4.0-4.6) 0.2 6	4.4 (3.9-4.8) 0.4 6	4.2 (3.9-4.6) 0.2 6
Ratio of Blood Urea Nitrogen to Creatinine	12.8 (12.6-13.1) 0.4 2	10.7 (6.8-13.6) 2.5 5	15.7 (11.7-24.9) 5.0 6	15.6 (11.8-22.3) 3.6 6	18.1 (14.5-19.8) 1.8 6
Ratio of Albumin to Globulin	2.9 (2.9-3.0) 0.1 2	3.4 (2.9-3.8) 0.4 5	3.5 (2.7-4.1) 0.6 6	3.6 (2.7-4.2) 0.6 6	2.5 (2.1-3.1) 0.4 6

* MEAN
(MIN-MAX)
STD
N

Table 5. Descriptive Statistics* of Hematology Parameters, by Study Day for the Six Animals Tested in Phase I, Part B.

Parameter	Study Day				
	Physical	0	1	3	7
Basophils (#/mL)	0.048 (0.030-0.082) 0.018 6	0.071 (0.026-0.128) 0.047 5	0.058 (0.002-0.147) 0.050 6	0.151 (0.096-0.261) 0.065 5	0.206 (0.161-0.269) 0.038 6
Eosinophils (#/mL)	0.038 (0.005-0.078) 0.032 6	0.072 (0.009-0.134) 0.057 5	0.029 (0.006-0.090) 0.032 6	0.179 (0.031-0.277) 0.101 5	0.194 (0.077-0.292) 0.079 6
Hematocrit (%)	29.850 (28.300-32.100) 1.365 6	32.060 (28.600-34.500) 2.327 5	30.400 (27.500-34.500) 2.498 6	31.180 (27.900-33.800) 2.519 5	29.667 (24.900-32.500) 2.796 6
Hemoglobin (g/dL)	10.032 (9.300-11.000) 0.587 6	10.690 (9.650-11.400) 0.714 5	10.237 (9.070-11.500) 0.830 6	10.146 (9.070-11.000) 0.931 5	9.568 (8.130-10.300) 0.833 6
Lymphocytes (#/mL)	3.350 (2.040-4.870) 1.055 6	4.338 (3.430-4.770) 0.531 5	3.888 (2.210-6.530) 1.579 6	5.862 (1.590-8.640) 2.623 5	5.853 (3.240-8.610) 2.129 6
Mean Corpuscular Hemoglobin (pg)	18.517 (17.400-19.200) 0.688 6	17.260 (16.300-18.300) 0.856 5	17.450 (16.300-18.200) 0.766 6	17.320 (15.900-18.000) 0.829 5	16.667 (15.200-17.400) 0.873 6
Mean Corpuscular Concentration (g/dL)	33.617 (32.900-34.400) 0.591 6	33.340 (32.700-34.400) 0.702 5	33.650 (32.600-35.500) 1.117 6	32.520 (31.500-33.200) 0.661 5	32.267 (31.700-32.600) 0.314 6
Mean Corpuscular Volume (fL)	55.117 (53.100-56.500) 1.440 6	51.800 (49.600-54.400) 1.771 5	51.883 (50.000-54.800) 1.716 6	53.260 (49.100-55.200) 2.387 5	51.783 (47.200-54.400) 2.914 6
Monocytes (#/mL)	1.078 (0.788-1.370) 0.211 6	1.324 (0.712-1.940) 0.502 5	1.040 (0.839-1.550) 0.309 6	2.076 (1.660-2.780) 0.419 5	2.503 (2.260-2.900) 0.236 6
Neutrophils (#/mL)	4.803 (2.390-8.660) 2.275 6	5.284 (2.980-7.120) 1.505 5	4.370 (2.310-6.330) 1.319 6	8.934 (5.810-13.400) 3.174 5	8.320 (6.850-9.440) 0.929 6

* MEAN
(MIN-MAX)
STD
N

Table 5. Descriptive Statistics* of Hematology Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Parameter	Study Day				
	Physical	0	1	3	7
Platelet Count (#/mL)	595.667 (505.000-697.000) 77.948 6	691.400 (573.000-846.000) 102.166 5	634.500 (513.000-862.000) 121.070 6	558.200 (436.000-714.000) 112.611 5	785.833 (624.000-989.000) 150.121 6
Red Blood Cell Count (#/mL)	5.415 (5.090-5.830) 0.261 6	6.210 (5.260-6.840) 0.628 5	5.857 (5.300-6.290) 0.377 6	5.852 (5.320-6.280) 0.377 5	5.728 (5.060-6.040) 0.378 6
White Blood Cell Count (#/mL)	9.303 (6.380-12.400) 2.274 6	11.090 (8.350-12.500) 1.604 5	9.355 (6.840-12.900) 2.353 6	17.200 (12.200-22.400) 4.693 5	17.083 (15.300-20.200) 1.975 6

* MEAN
(MIN-MAX)
STD
N

Table 6. Descriptive Statistics* of Thiodiglycol Parameters, by Study Day for the Six Animals Tested in Phase I, Part B.

Study Day	Urine Volume (mL)	Thiodiglycol Concentration (µg/mL)
0 (AM)	94.83 (17.00-220.00) 70.56 6	0.00 (0.00-0.00) 0.00 6
0 (PM)	175.17 (21.00-483.00) 177.47 6	2.14 (0.66-4.98) 1.66 6
1 (AM)	36.17 (0.00-71.00) 24.15 6	1.57 (0.09-3.16) 1.11 5
1 (PM)	210.20 (67.00-369.00) 112.85 5	0.27 (0.05-1.05) 0.44 5
2 (AM)	103.75 (6.50-220.00) 85.80 6	0.65 (0.15-1.94) 0.71 6
2 (PM)	194.40 (58.00-356.00) 144.72 5	0.21 (0.06-0.43) 0.16 5
3 (AM)	95.83 (14.00-190.00) 67.69 6	0.18 (0.07-0.38) 0.12 6
3 (PM)	134.40 (46.00-337.00) 117.99 5	0.05 (0.00-0.11) 0.05 5

* MEAN
(MIN-MAX)
STD
N

Table 6. Descriptive Statistics* of Thiodiglycol Parameters, by Study Day for the Six Animals Tested in Phase I, Part B (Continued).

Study Day	Urine Volume (mL)	Thiodiglycol Concentration (µg/mL)
4 (AM)	71.00 (20.00-115.00) 37.00 6	0.04 (0.00-0.13) 0.05 6
4 (PM)	189.67 (78.00-358.00) 99.80 6	0.03 (0.00-0.06) 0.02 6
5 (AM)	78.83 (36.00-127.00) 30.80 6	0.04 (0.00-0.12) 0.05 6
5 (PM)	181.33 (72.00-371.00) 104.41 6	0.02 (0.00-0.12) 0.05 6
6 (AM)	114.33 (64.00-198.00) 49.56 6	0.02 (0.00-0.08) 0.03 6
6 (PM)	201.33 (85.00-453.00) 143.41 6	0.01 (0.00-0.05) 0.02 6
7 (AM)	185.83 (72.00-318.00) 91.06 6	0.03 (0.00-0.08) 0.04 6
7 (PM)	247.50 (165.00-339.00) 87.86 4	0.01 (0.00-0.03) 0.02 4

* MEAN
(MIN-MAX)
STD
N

Table 7. Distributions of Urinalysis Results for Each Test, by Study Day for the Six Animals Tested in Phase I, Part B.

Test	Result	Percent of samples															
		Study Day															
		0 (AM)	0 (PM)	1 (AM)	1 (PM)	2 (AM)	2 (PM)	3 (AM)	3 (PM)	4 (AM)	4 (PM)	5 (AM)	5 (PM)	6 (AM)	6 (PM)	7 (AM)	7 (PM)
Glucose (mg/dL)	negative	100	100	100	67	83	100	100	100	100	100	100	100	100	100	100	100
	100				17												
	1000				17												
	2000					17											
Bilirubin	negative	100	100	100	100	100	100	100	100	100	100	83	83	100	100	100	100
	small											17	17				
Ketone (mg/dL)	negative	100	100	100	100	100	100	83	100	100	100	100	100	100	100	83	100
	5							17								17	
Blood	negative	50		60	17	17		33						33		50	17
	trace	17	33		17	17						67		33	17	33	33
	small	17	17			33	33			33	67	17	67	17	50	17	33
	moderate		17	20	17	33		17	17	33	33	17	33	17	33		17
	large	17	33	20	50		67	50	83	33							
Protein (mg/dL)	negative	67	33		17	17	17		50		17				17		17
	trace	33	17	60	83	67	67	33	33	33	33	33	33	33	33	67	67
	30		33	40			17	67	17	67	50	67	67	67	50	33	17
	100					17											
	2000		17														
Nitrate	negative	83	100	80	67	67	67	33	33	33	33	17	50	33	33	17	
	positive	17		20	33	33	33	67	67	67	67	83	50	67	67	83	100

Table 7. Distributions of Urinalysis Results for Each Test, by Study Day for the Six Animals Tested in Phase I, Part B (Continued)

Test	Result	Percent of samples															
		Study Day															
		0 (AM)	0 (PM)	1 (AM)	1 (PM)	2 (AM)	2 (PM)	3 (AM)	3 (PM)	4 (AM)	4 (PM)	5 (AM)	5 (PM)	6 (AM)	6 (PM)	7 (AM)	7 (PM)
Leukocytes	negative	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
pH	5					33				17	33						
	6		33	40	17				17	50	17		17				17
	6.5	17		20	33	33	33	17	17	17							17
	7	17	17	40	33	17	50	33	50		17	33		17		33	17
	7.5	33	33		17	17		17	17		17	33	50	50	67	50	17
	8						17					17	17	17	33		
	8.5	33	17					33		17	17	17	17	17		17	33
Specific Gravity(mL)	1		17				17					17					
	1.005	50	17	20	17	17	17									17	
	1.01	33	33		33	17	33		17	33	17	17	33				33
	1.015	17					17	17		17	33		17	17	50	33	33
	1.02		17	20	33	33		50	50		17	50	17	33	33	33	17
	1.025			20	17	17	17			17			17	50			17
	1.03		17	40		17		33	33	33	33	17	17		17	17	

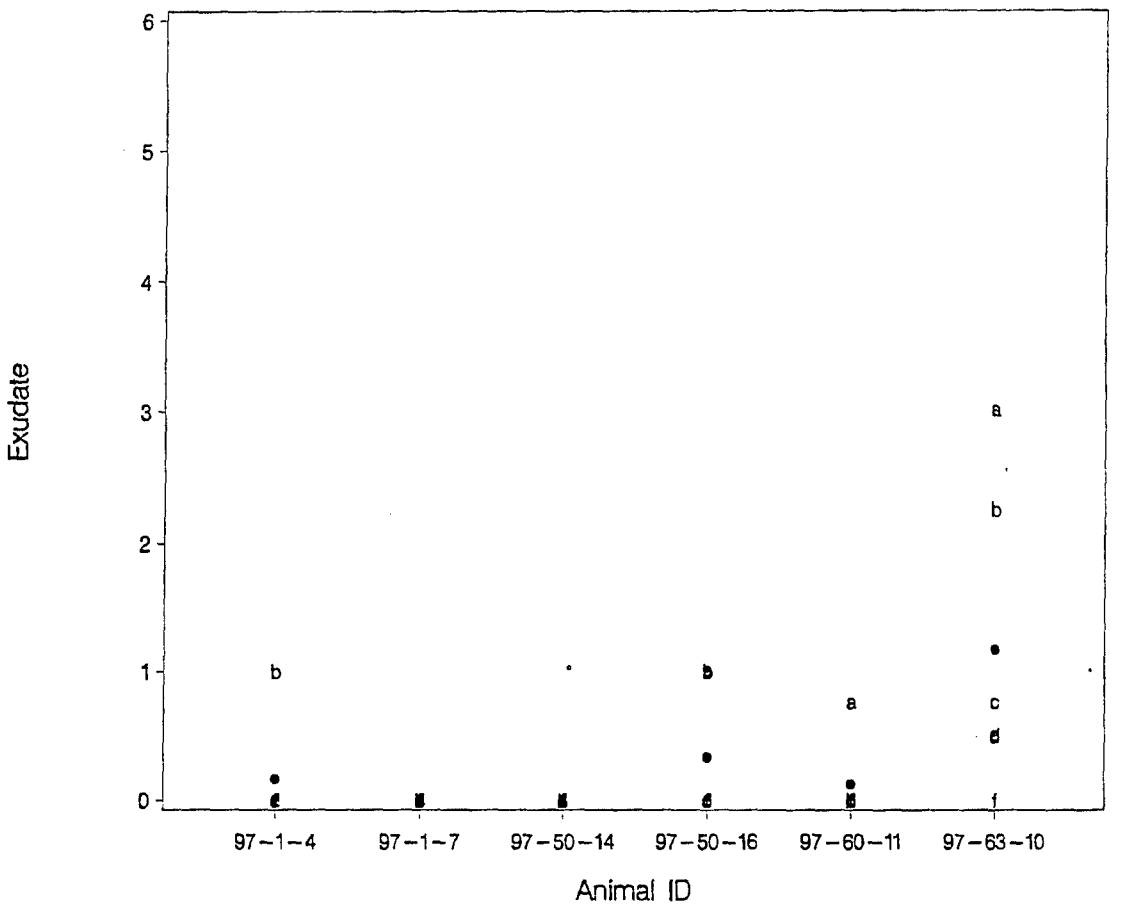

Figure 1. Clinical Observation Exudate of Six Different Animals on Study Day 2 in Phase I, Part B. Mean Exudate (•) Overlaid on Observed Values for Sites A-F.

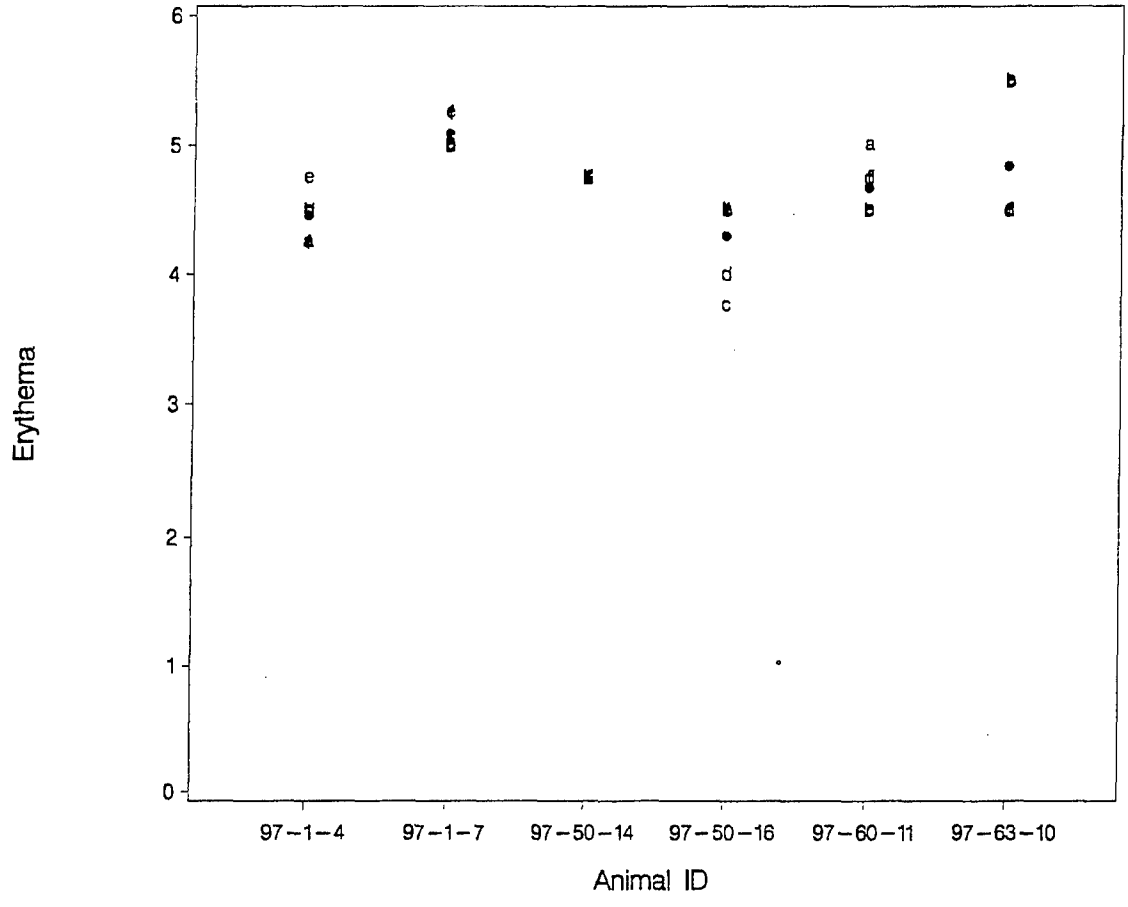

Figure 2. Clinical Observation Erythema of Six Different Animals on Study Day 2 in Phase I, Part B. Mean Erythema (•) Overlaid on Observed Values for Sites A-F.

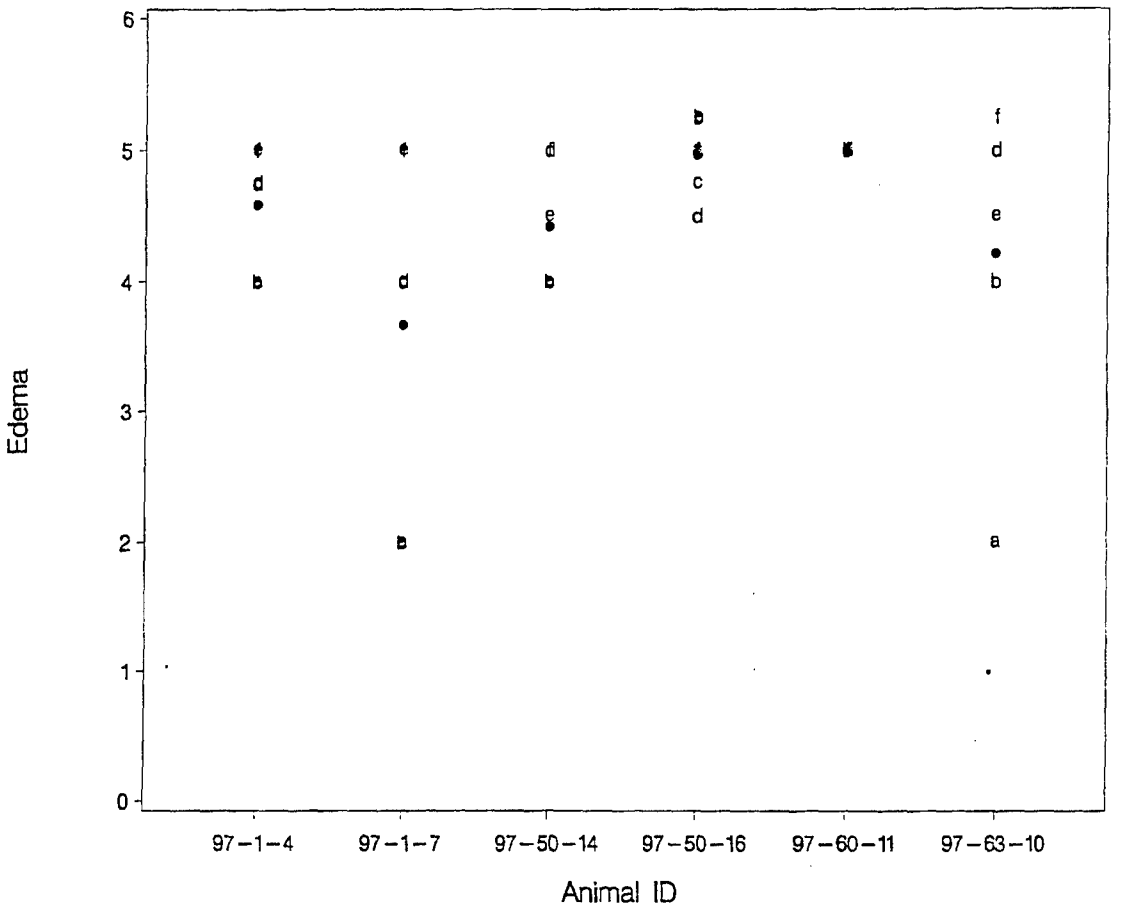

Figure 3. Clinical Observation Edema of Six Different Animals on Study Day 2 in Phase I, Part B. Mean Edema (•) Overlaid on Observed Values for Sites A-F.

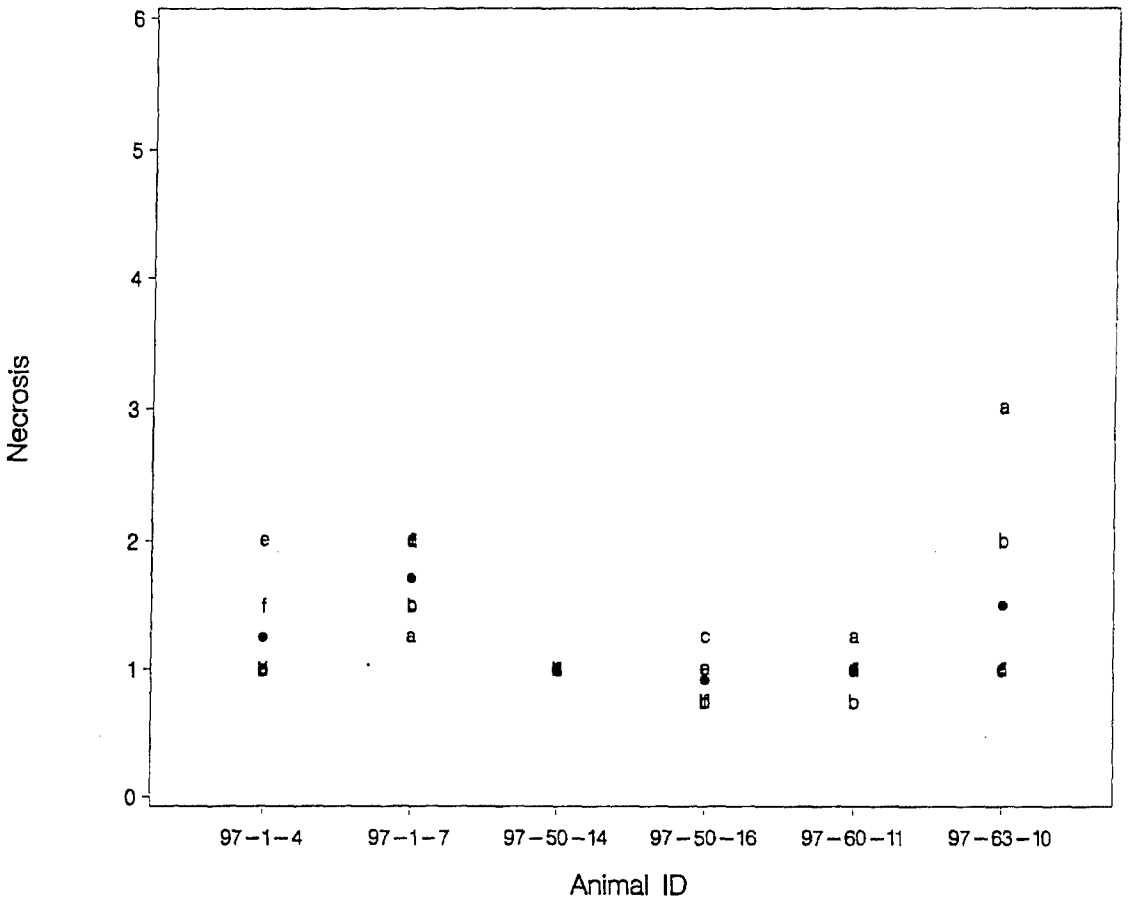

Figure 4. Clinical Observation Necrosis of Six Different Animals on Study Day 2 in Phase I, Part B. Mean Necrosis (•) Overlaid on Observed Values for Sites A-F.

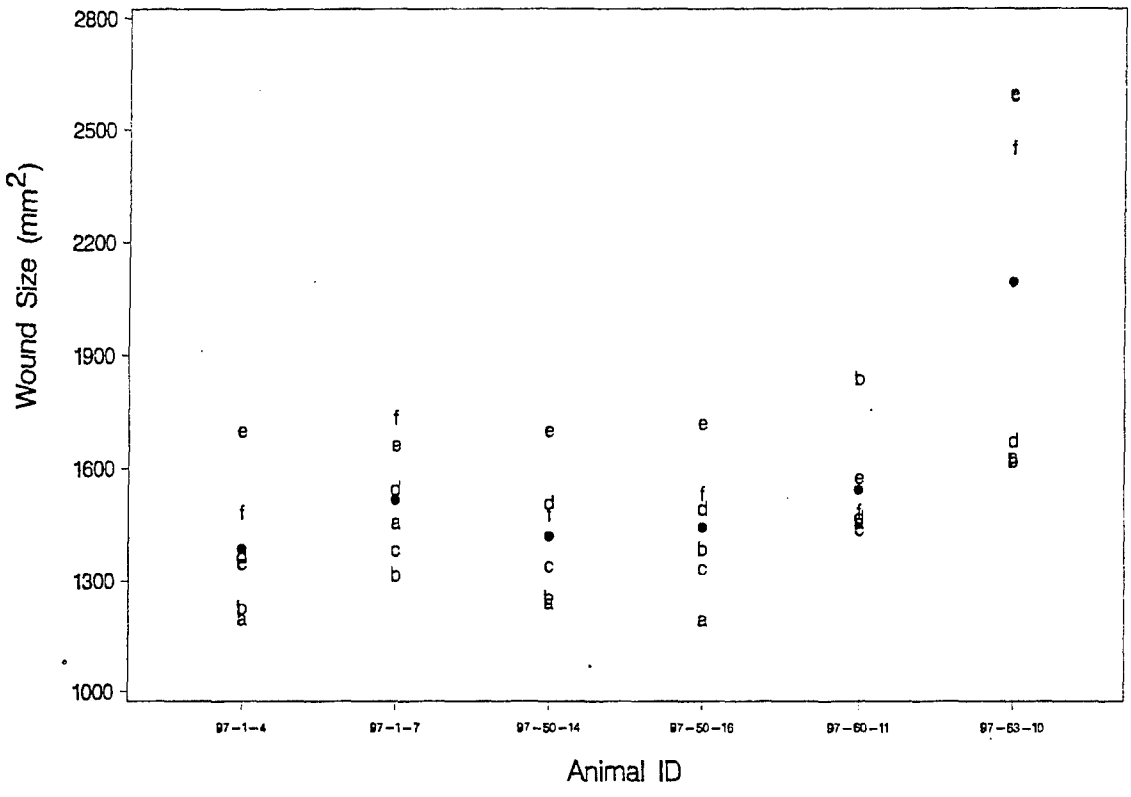

Figure 5. Wound Size (WS) of Six Different Animals on Study Day 2 in Phase I, Part B. Mean WS (•) Overlaid on Observed Values for Sites A-F.

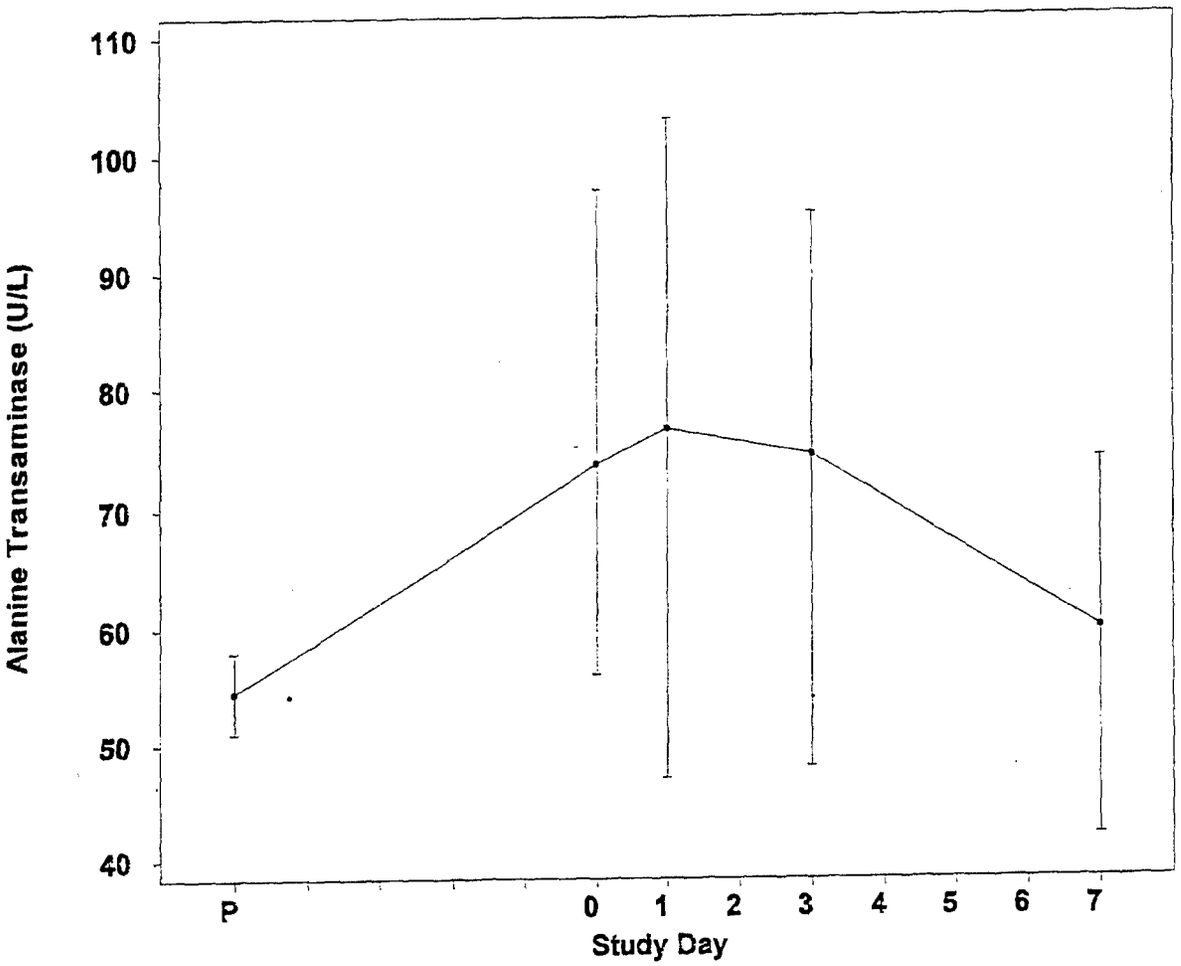

Figure 6. Mean, Minimum, and Maximum Alanine Transaminase (U/L) by Study Day for the Six Animals Tested in Phase I, Part B.

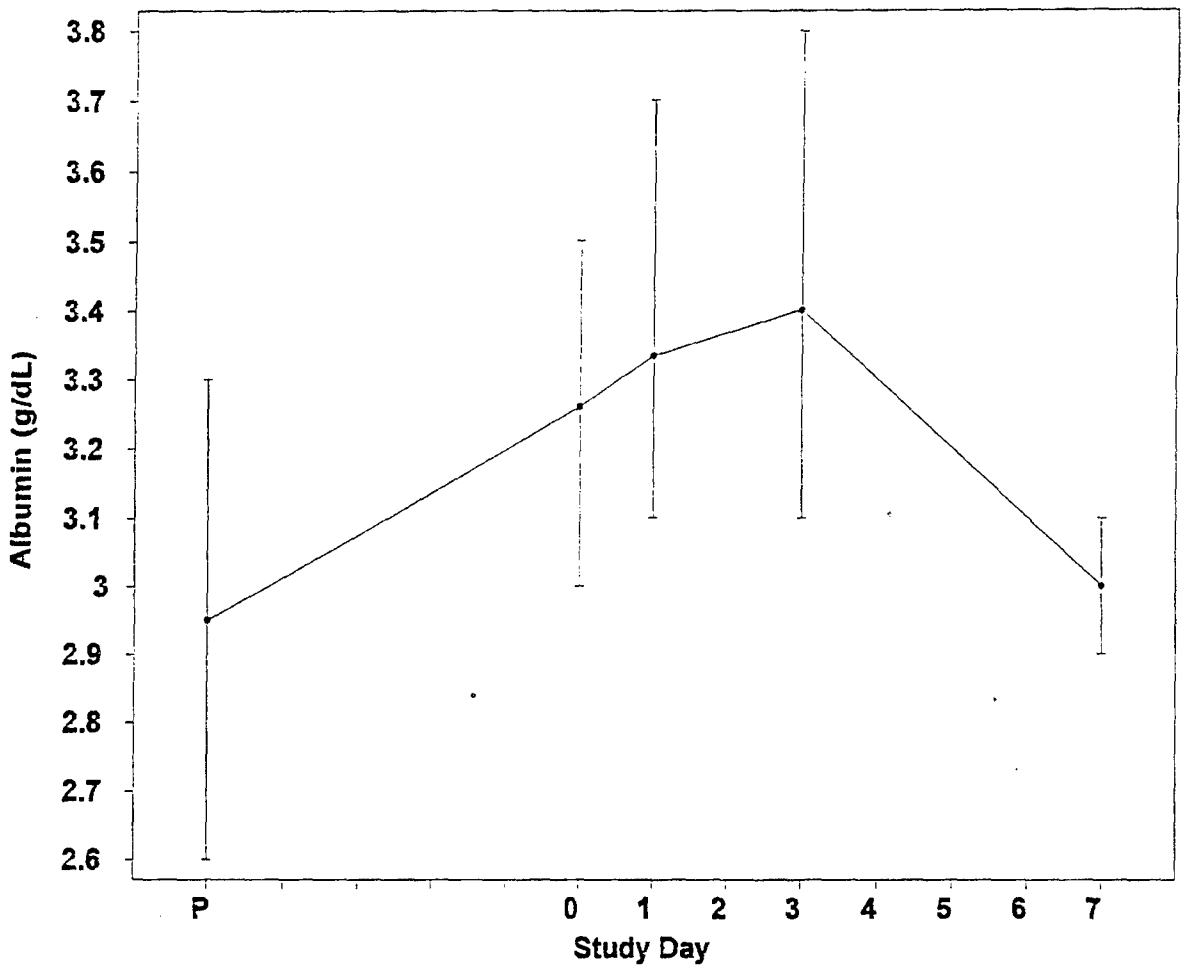

Figure 7. Mean, Minimum, and Maximum Albumin (g/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

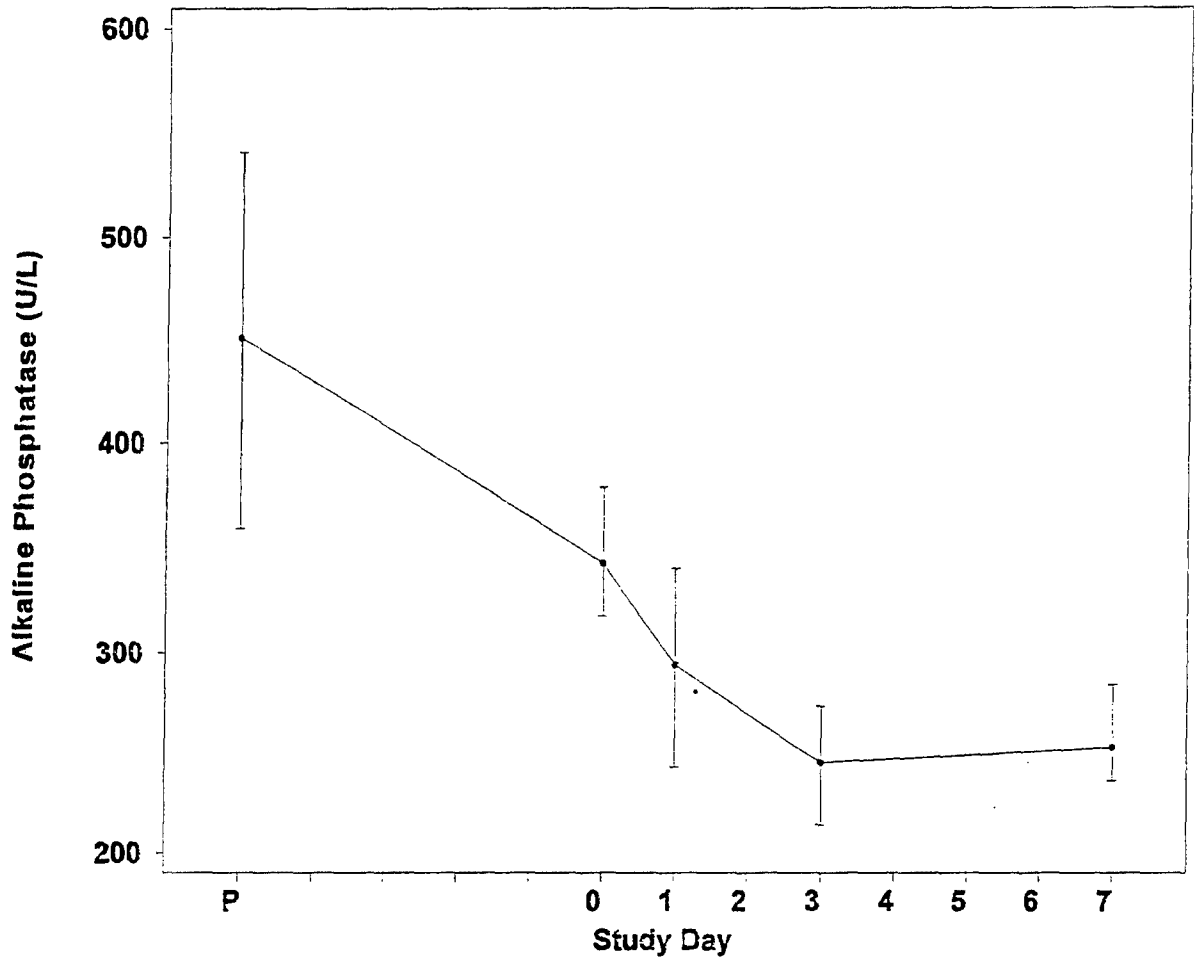

Figure 8. Mean, Minimum, and Maximum Alkaline Phosphatase (U/L) by Study Day for the Six Animals Tested in Phase I, Part B.

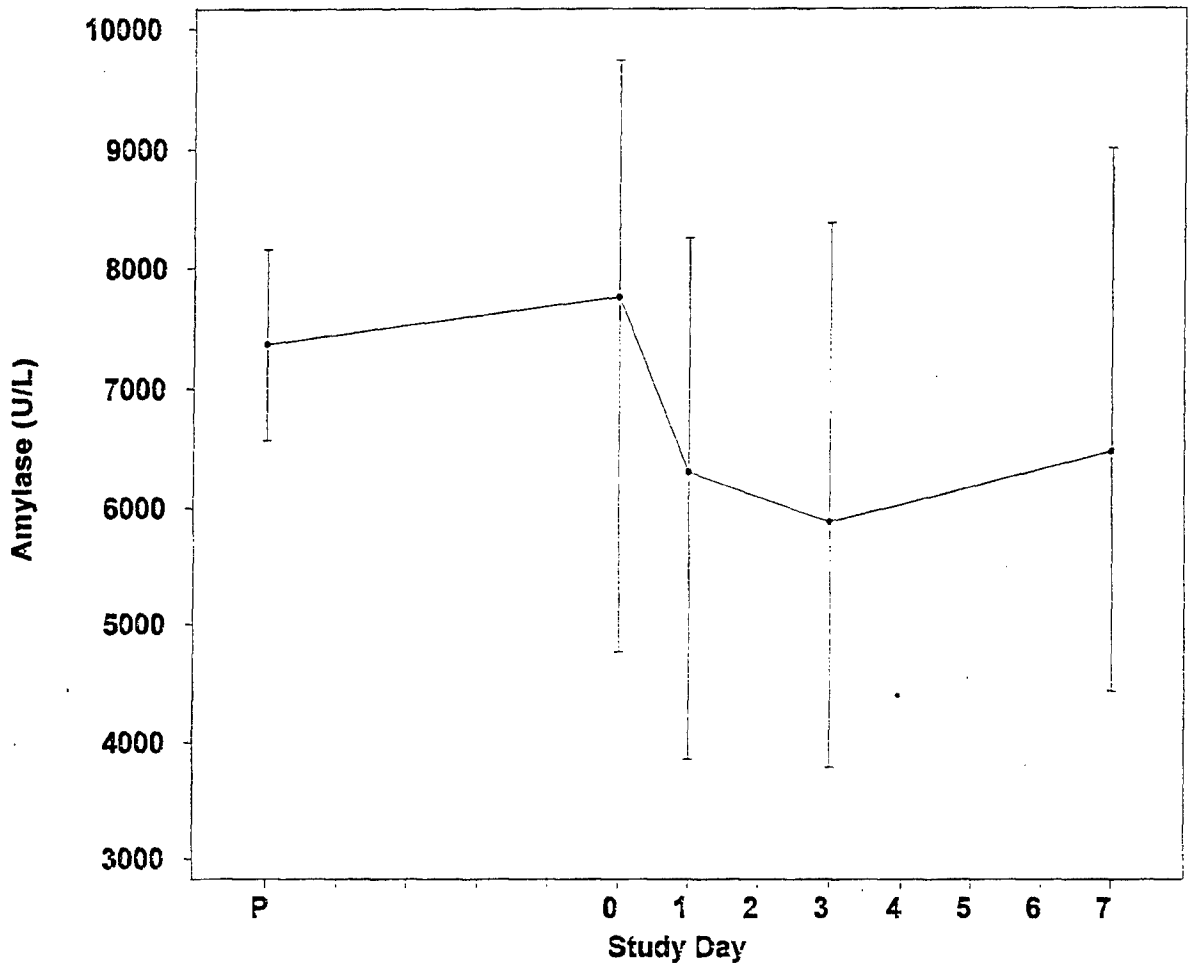

Figure 9. Mean, Minimum, and Maximum Amylase (U/L) by Study Day for the Six Animals Tested in Phase I, Part B.

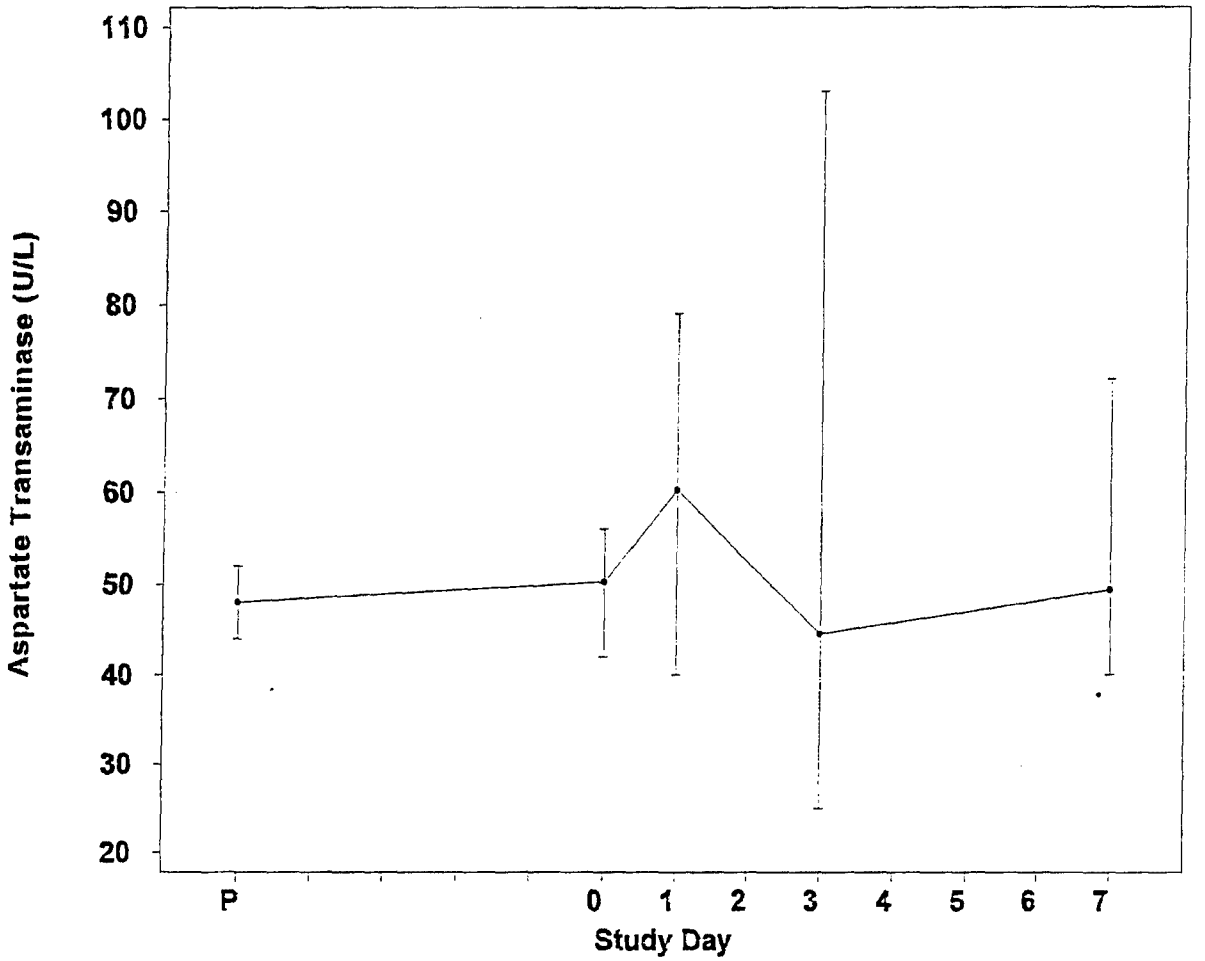

Figure 10. Mean, Minimum, and Maximum Aspartate Transaminase (U/L) by Study Day for the Six Animals Tested in Phase I, Part B.

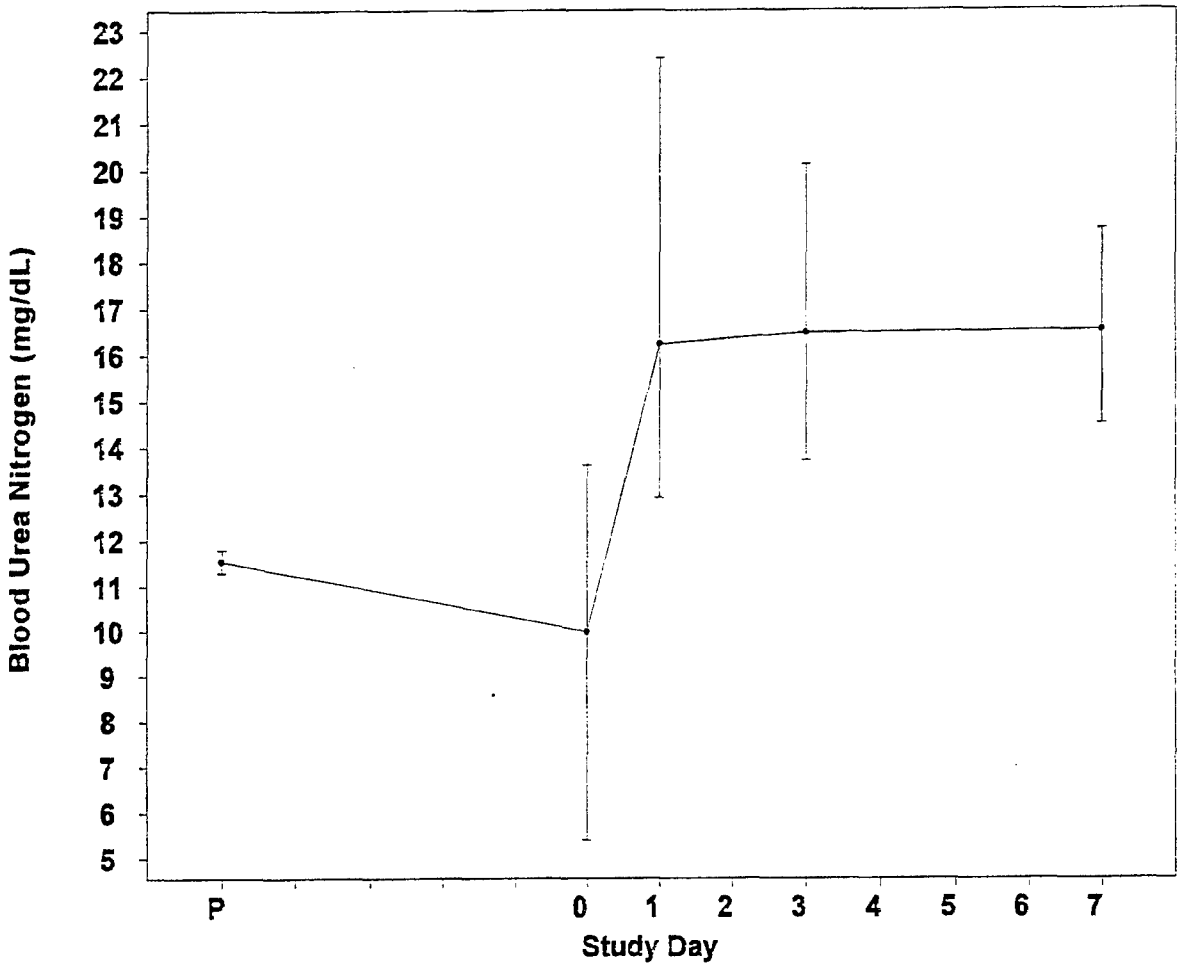

Figure 11. Mean, Minimum, and Maximum Blood Urea Nitrogen (mg/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

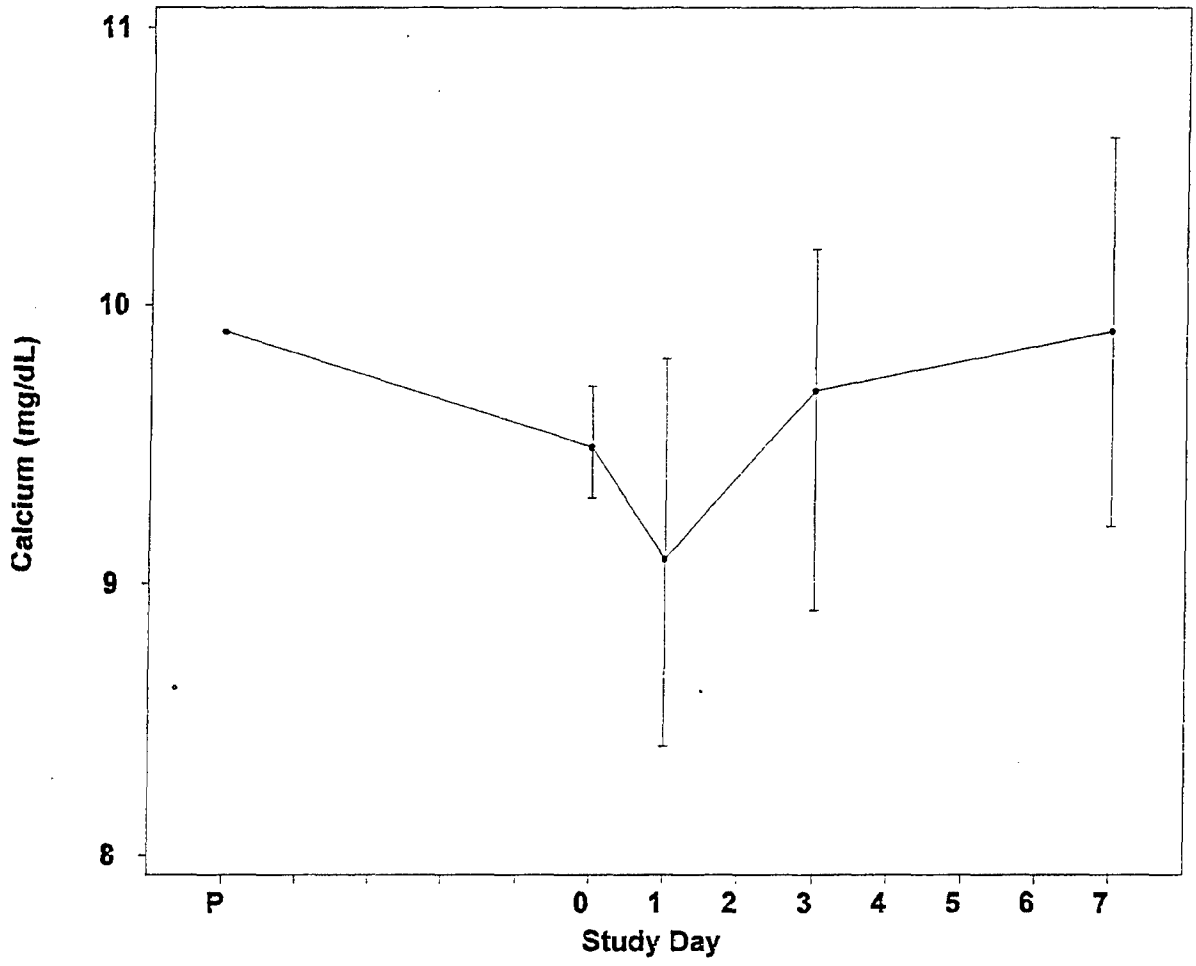

Figure 12. Mean, Minimum, and Maximum Calcium (mg/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

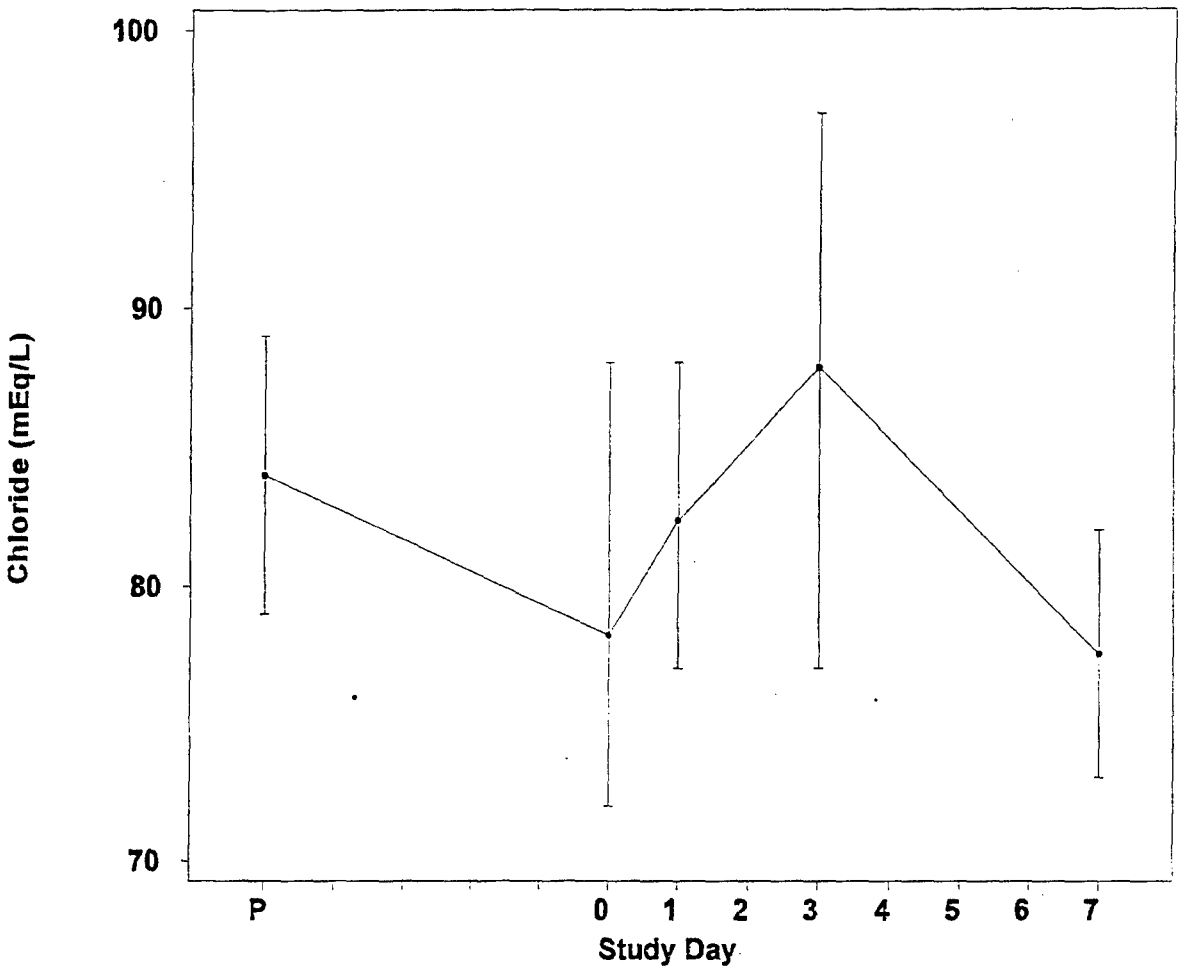

Figure 13. Mean, Minimum, and Maximum Chloride (mEq/L) by Study Day for the Six Animals Tested in Phase I, Part B.

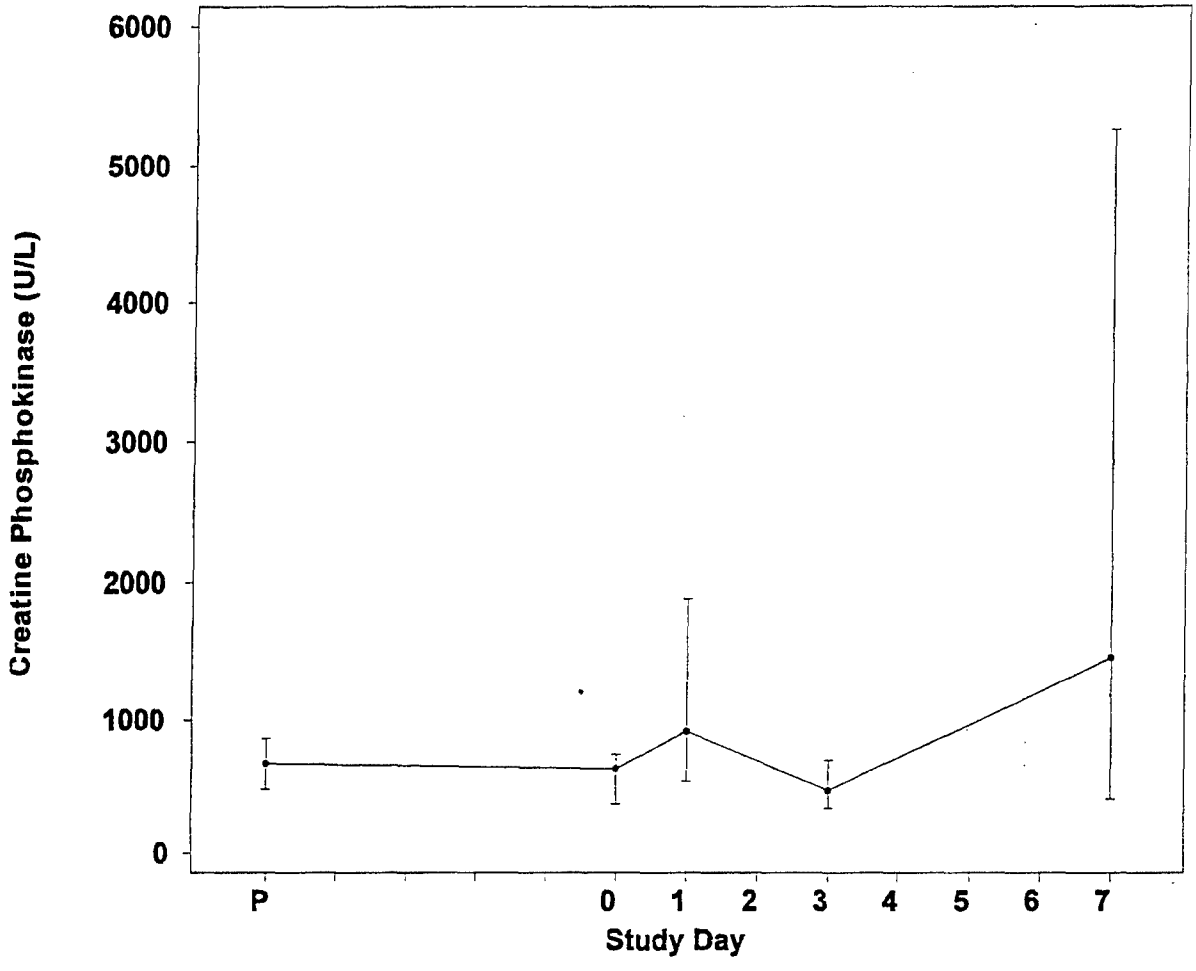

Figure 14. Mean, Minimum, and Maximum Creatine Phosphokinase (U/L) by Study Day for the Six Animals Tested in Phase I, Part B.

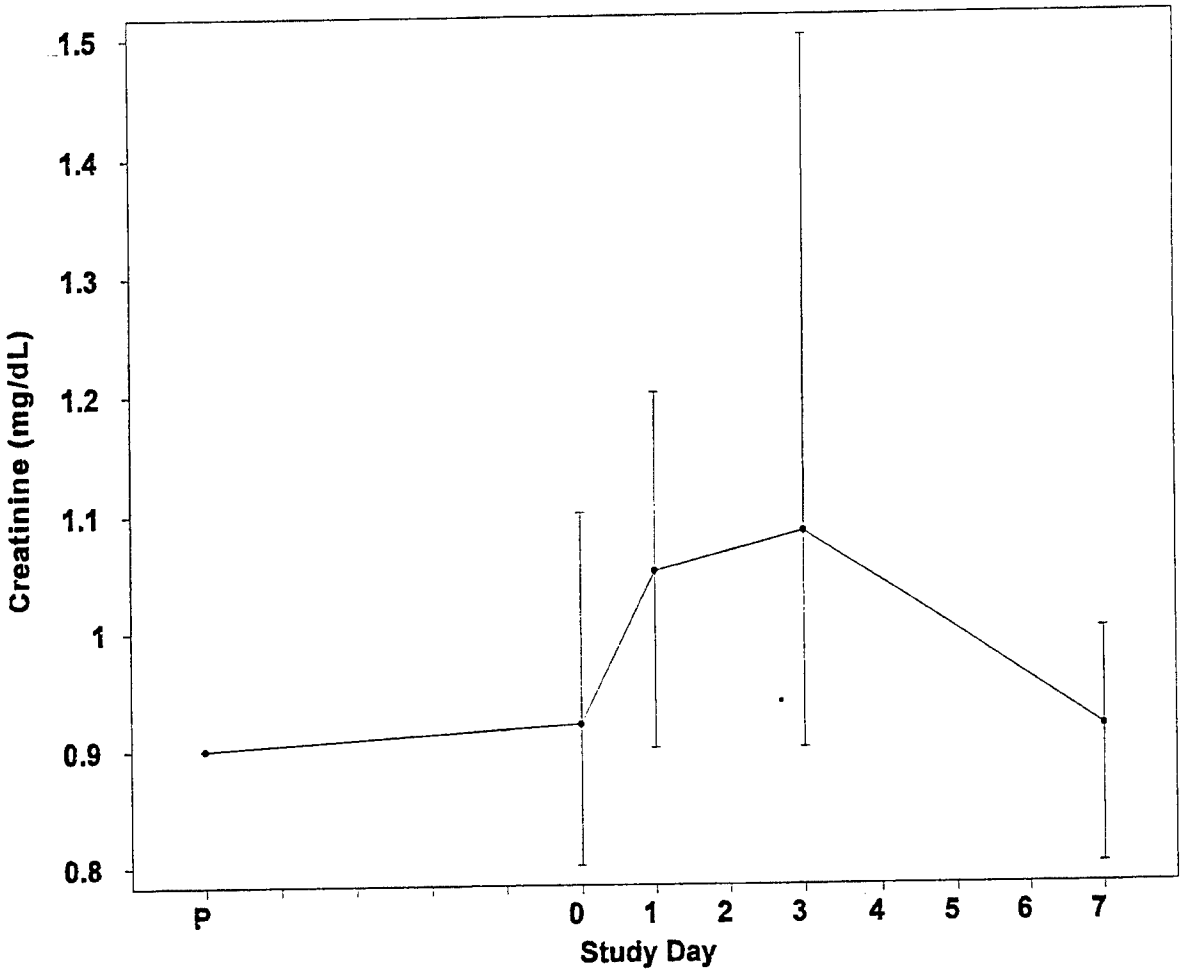

Figure 15. Mean, Minimum, and Maximum Creatinine (mg/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

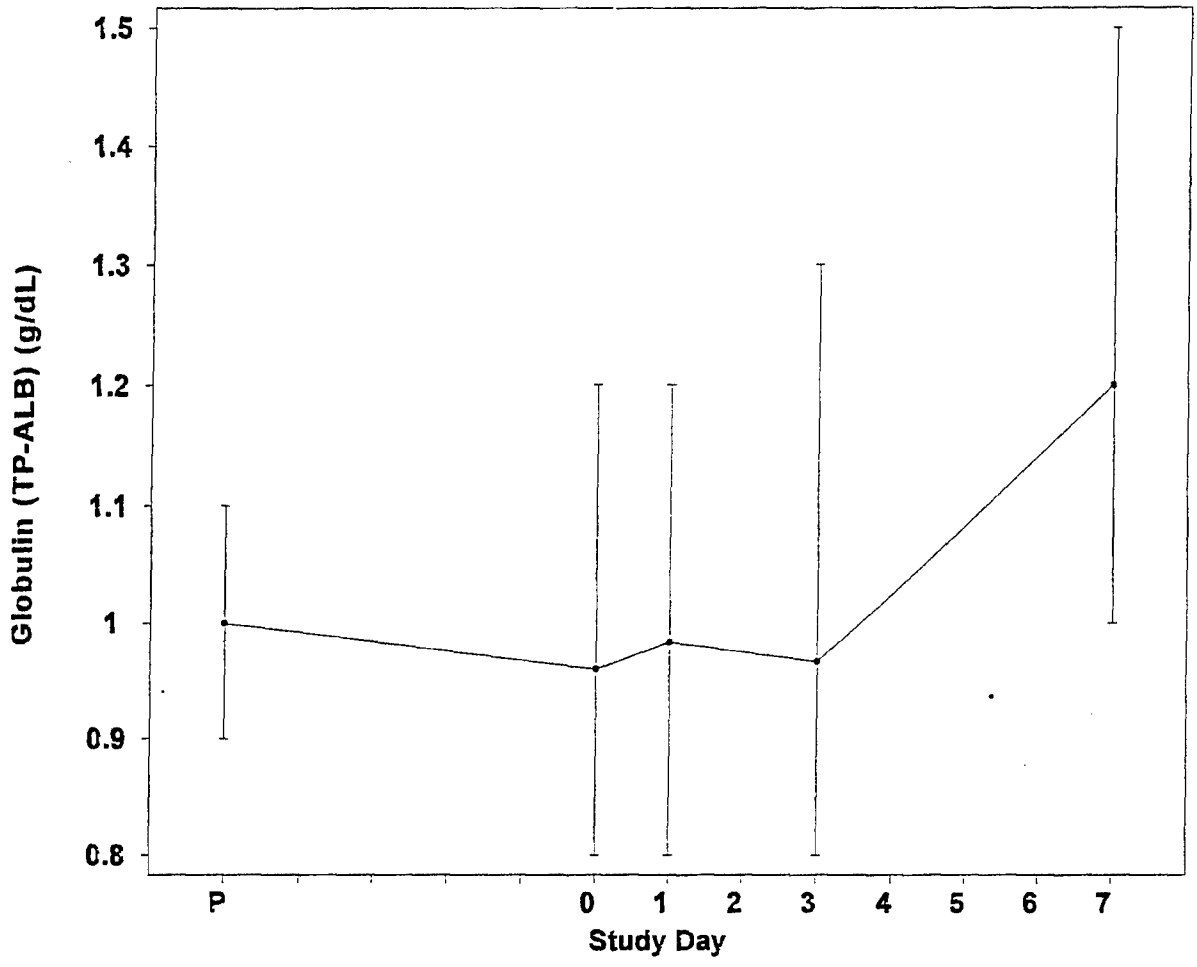

Figure 16. Mean, Minimum, and Maximum Globulin (TP-ALB) (g/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

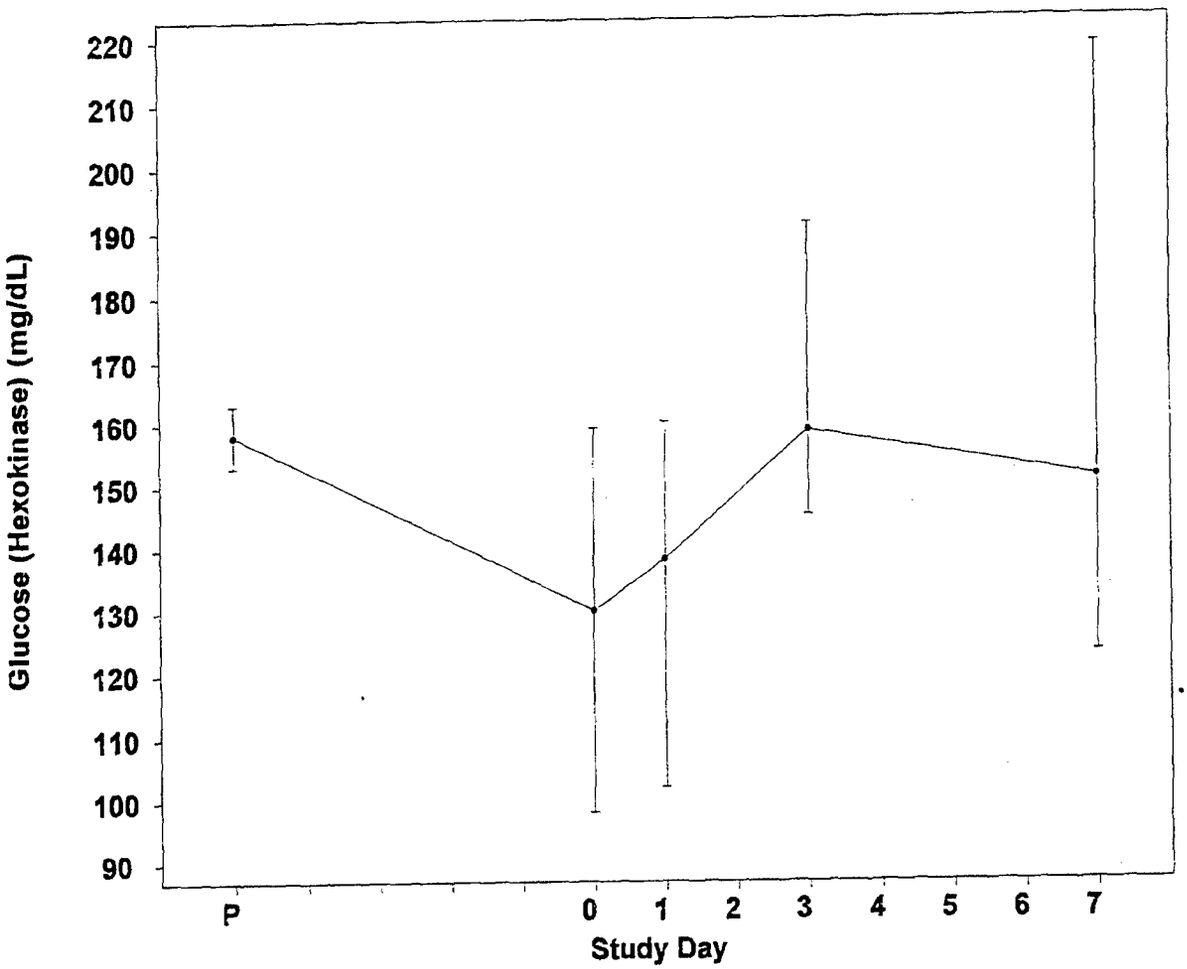

Figure 17. Mean, Minimum, and Maximum Glucose (Hexokinase) (mg/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

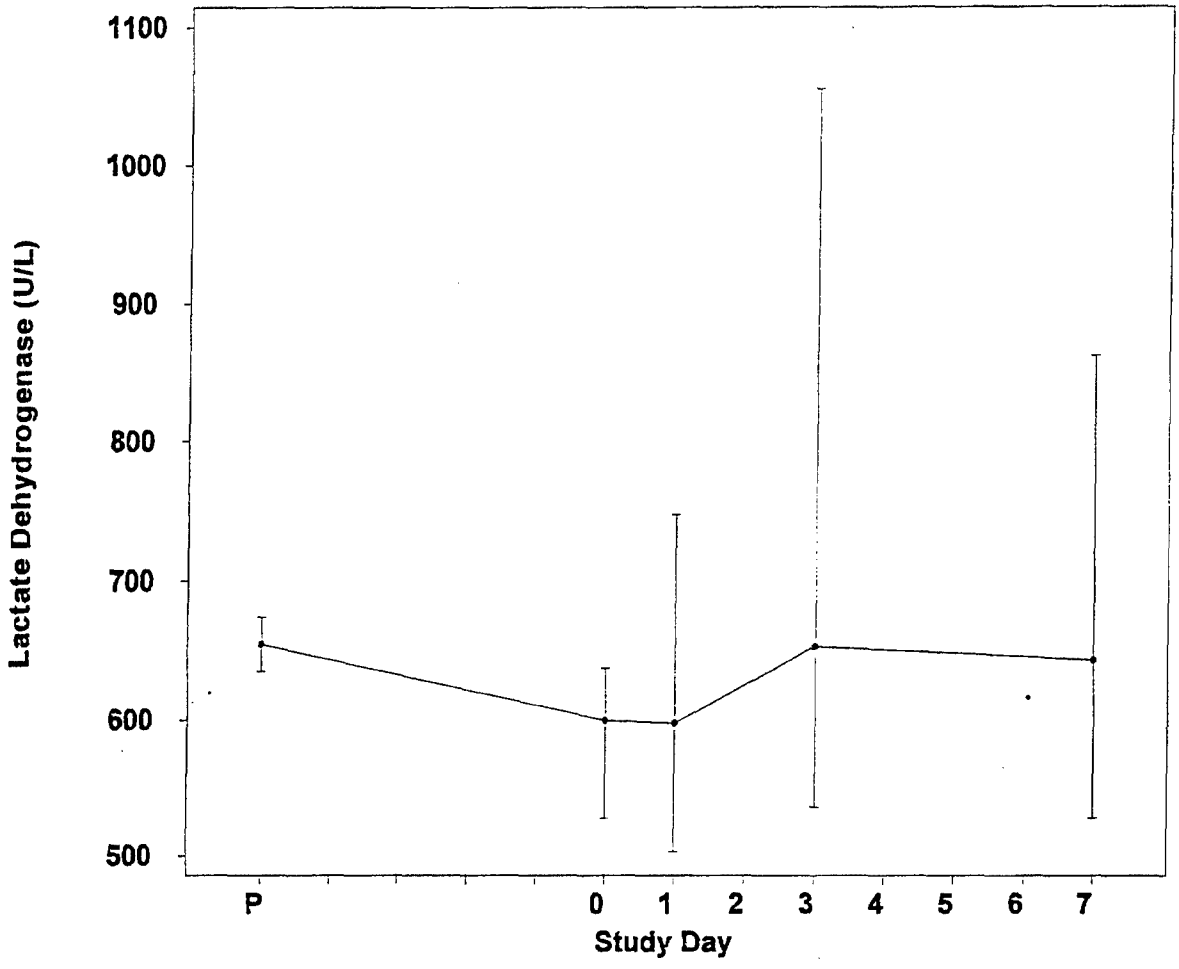

Figure 18. Mean, Minimum, and Maximum Lactate Dehydrogenase (U/L) by Study Day for the Six Animals Tested in Phase I, Part B.

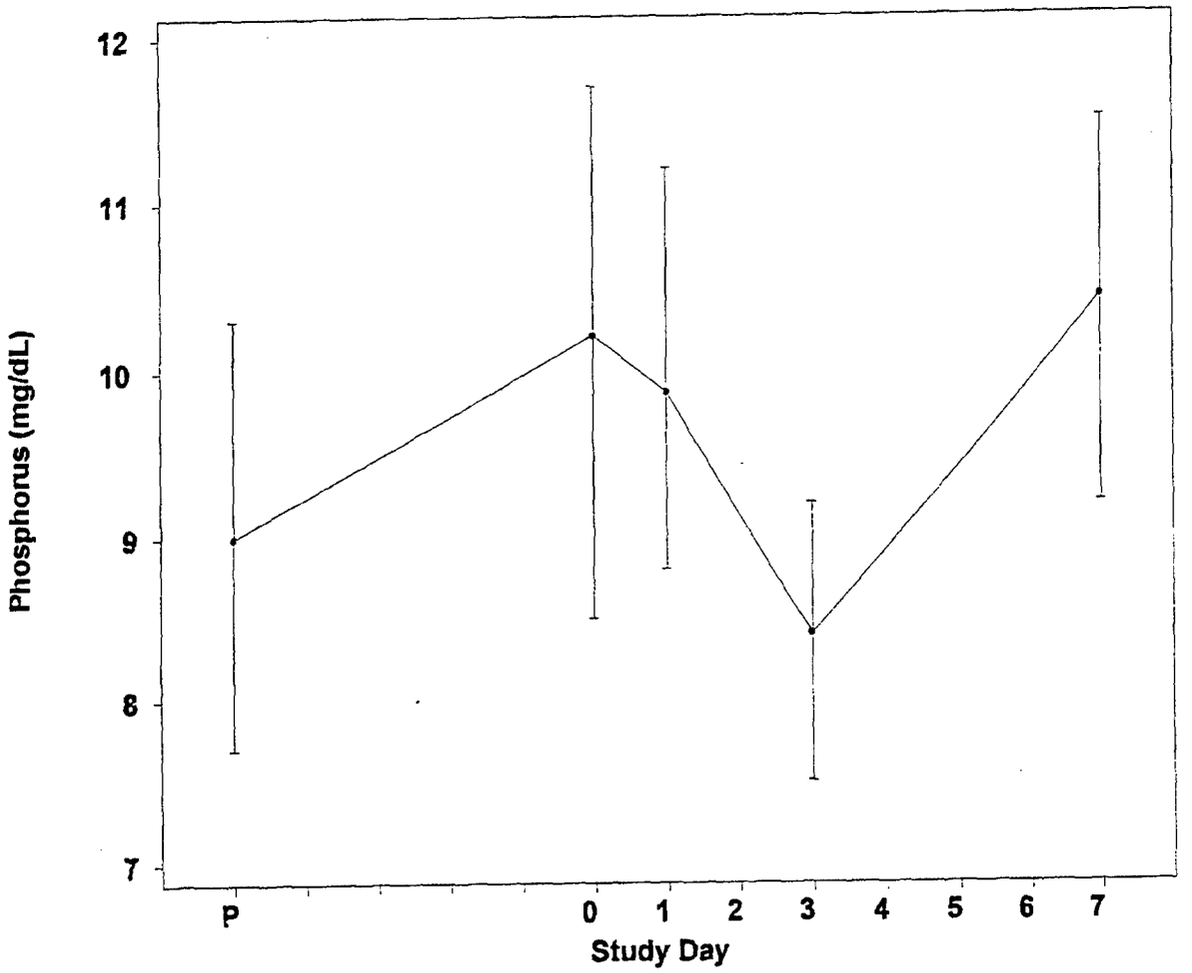

Figure 19. Mean, Minimum, and Maximum Phosphorus (mg/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

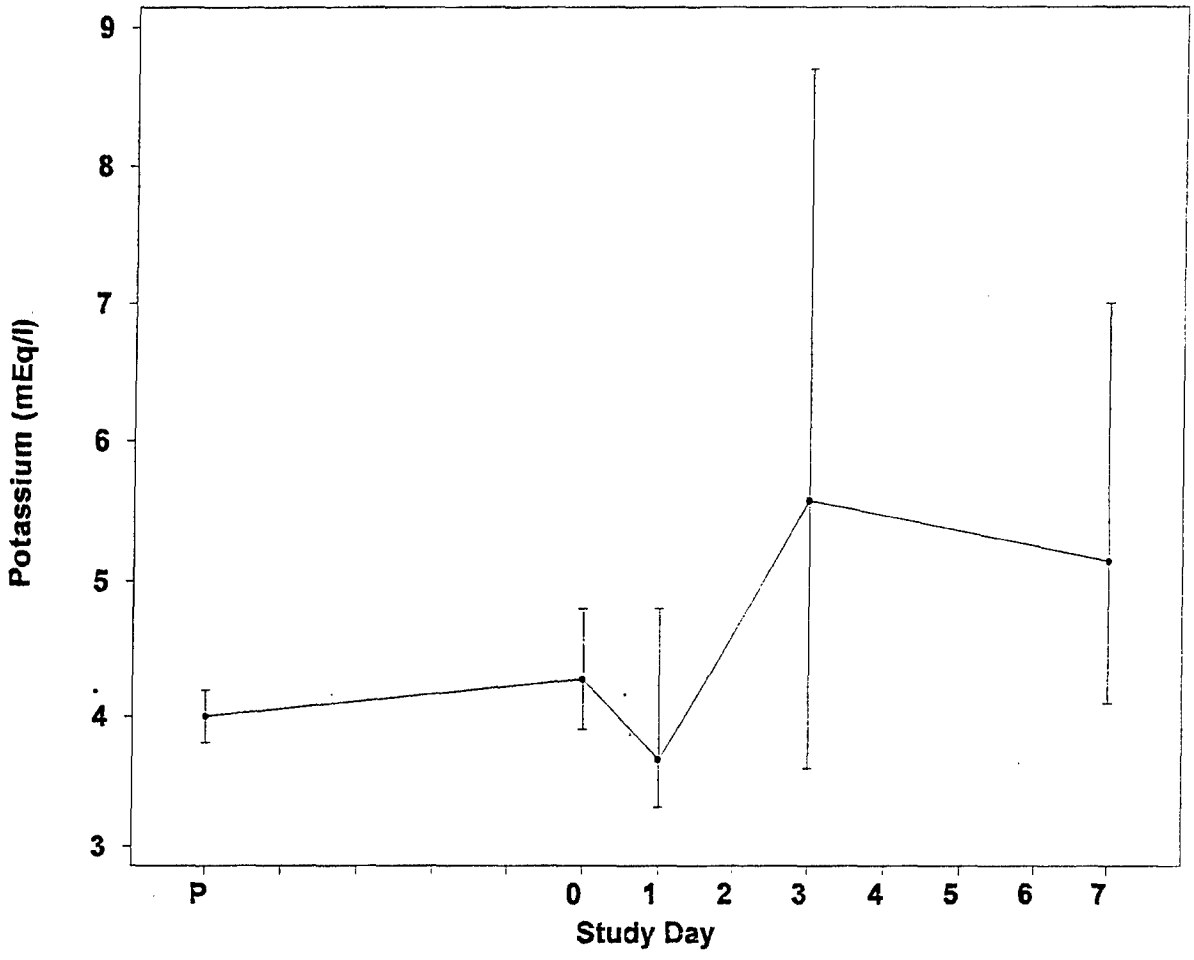

Figure 20. Mean, Minimum, and Maximum Potassium (mEq/l) by Study Day for the Six Animals Tested in Phase I, Part B.

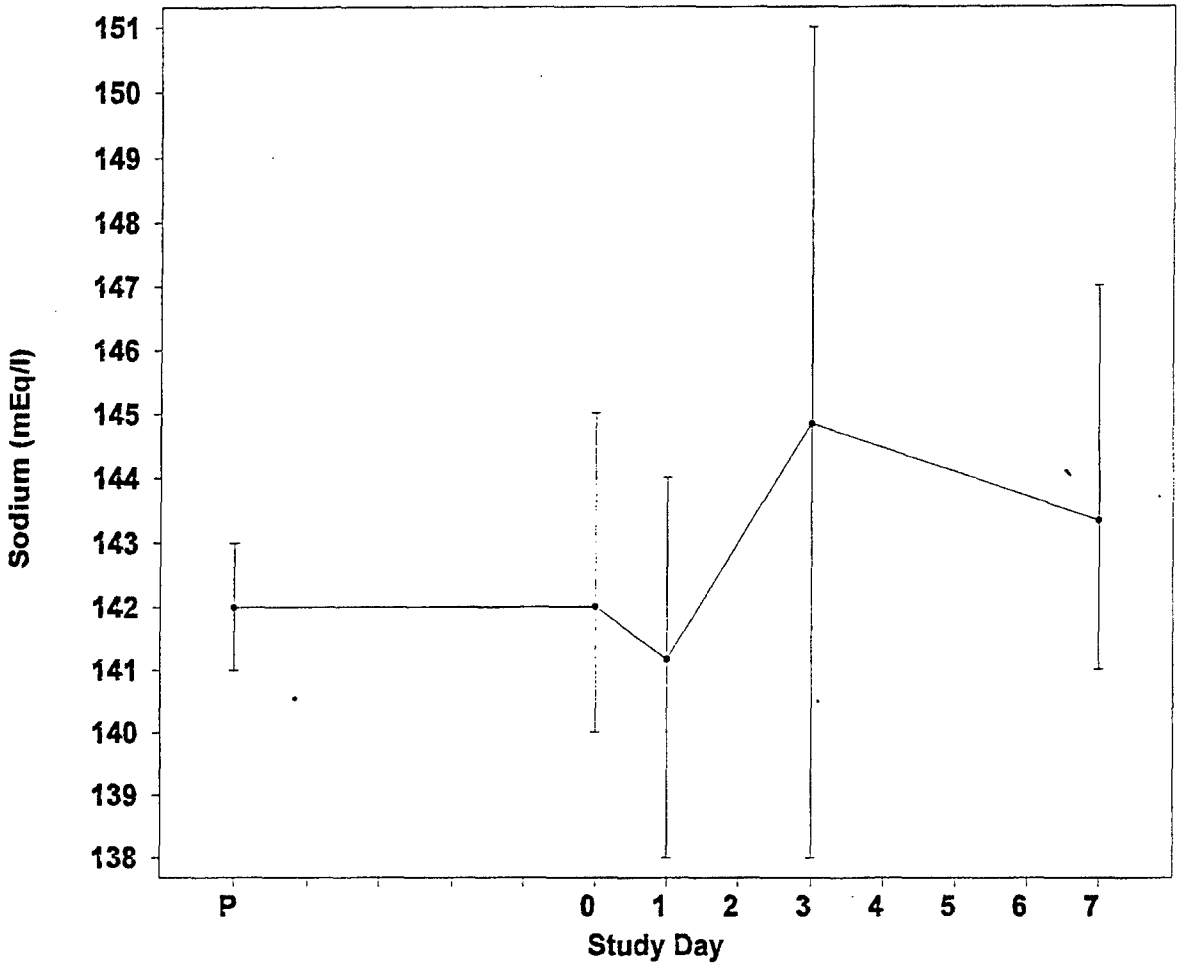

Figure 21. Mean, Minimum, and Maximum Sodium (mEq/l) by Study Day for the Six Animals Tested in Phase I, Part B.

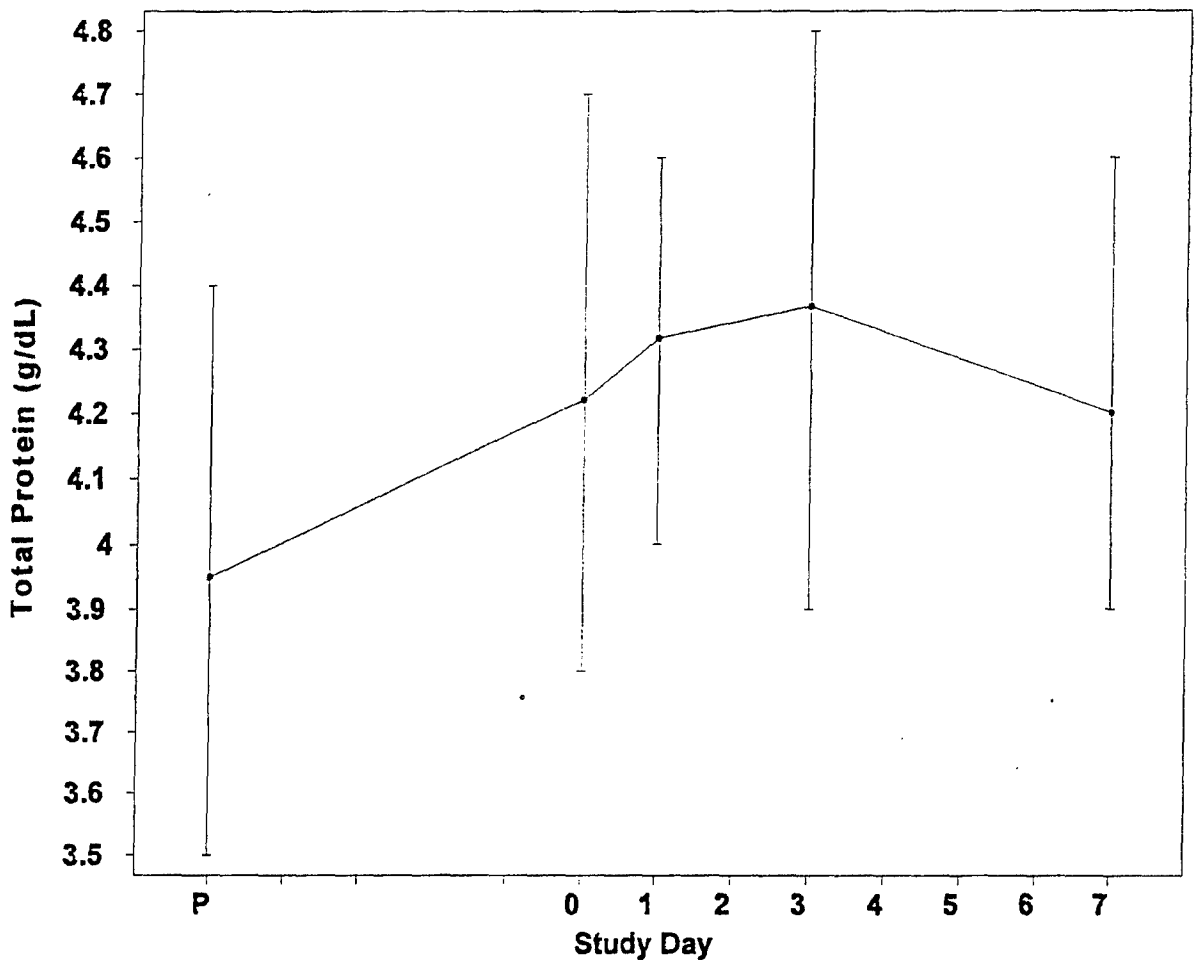

Figure 22. Mean, Minimum, and Maximum Total Protein (g/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

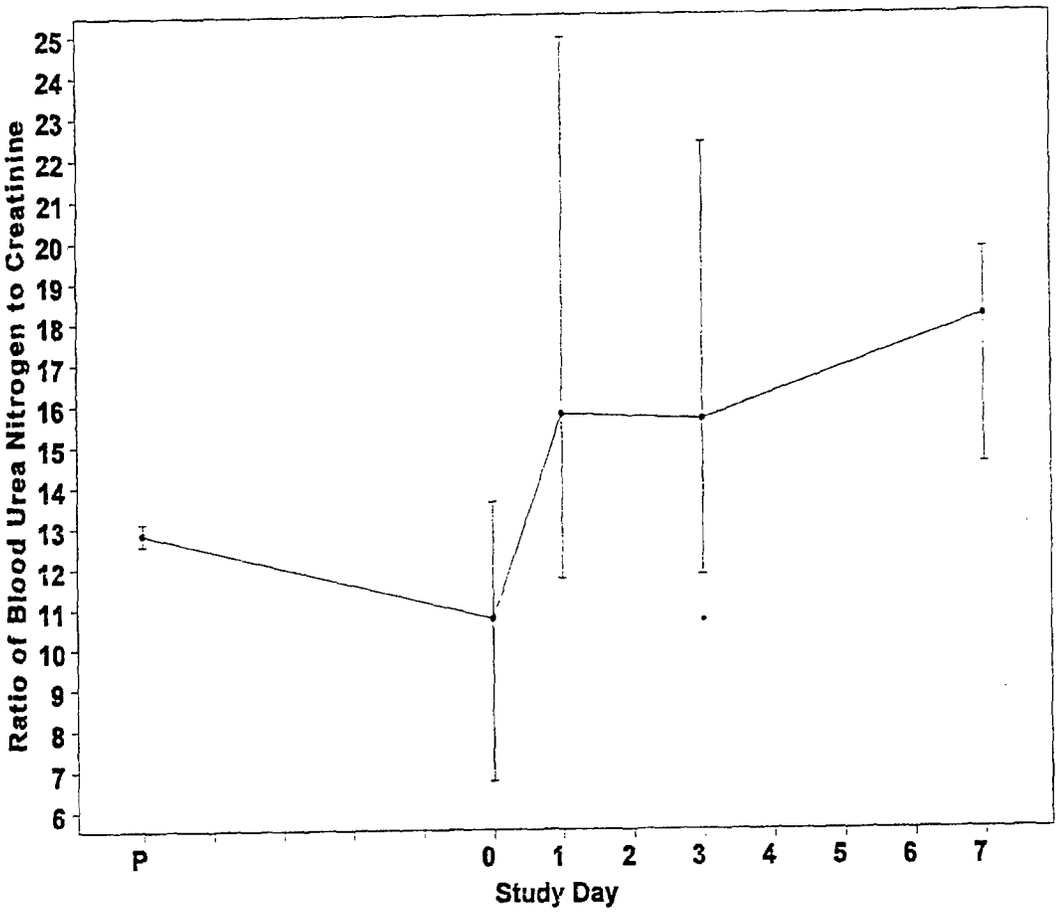

Figure 23. Mean, Minimum, and Maximum Ratio of Blood Urea Nitrogen to Creatinine by Study Day for the Six Animals Tested in Phase I, Part B.

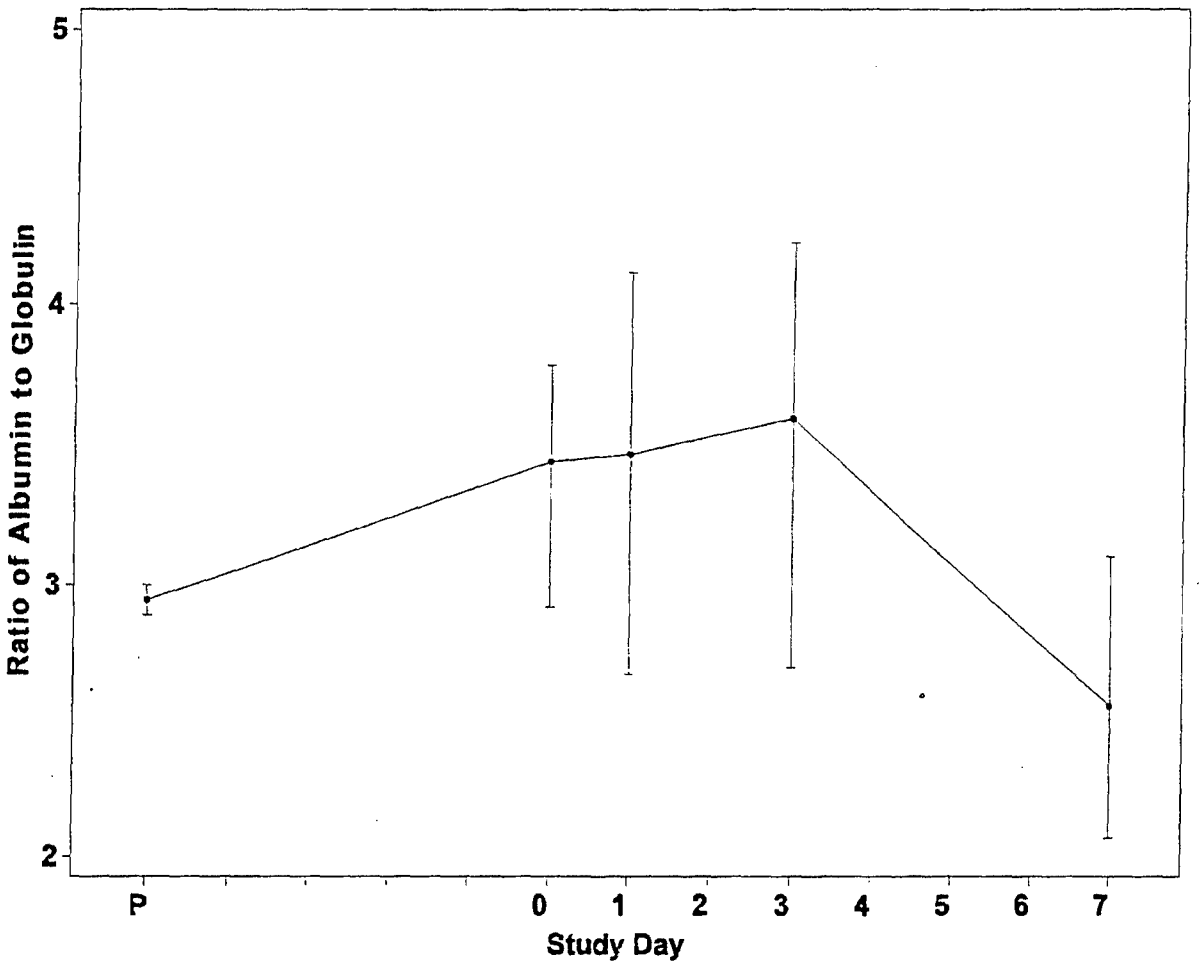

Figure 24. Mean, Minimum, and Maximum Ratio of Albumin to Globulin by Study Day for the Six Animals Tested in Phase I, Part B.

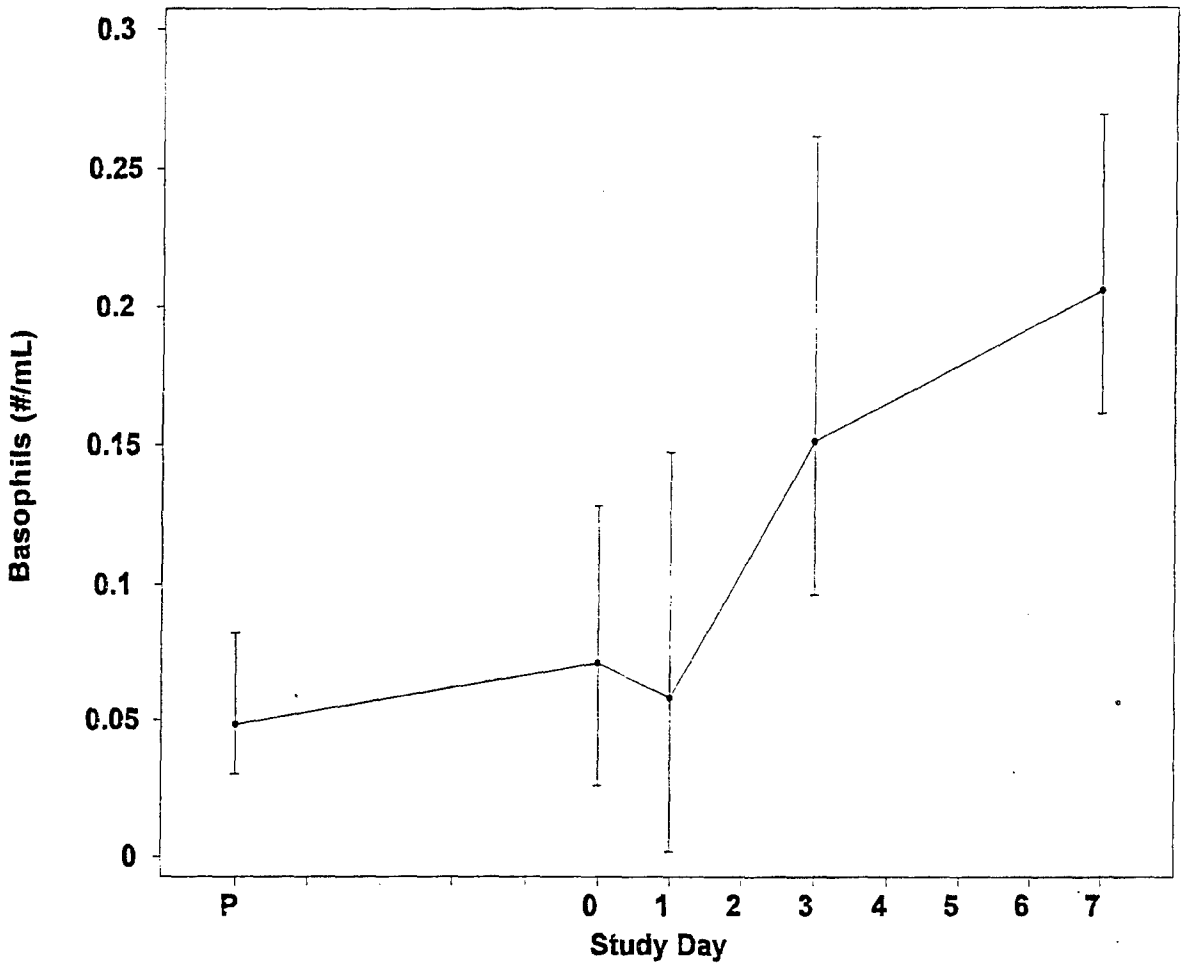

Figure 25. Mean, Minimum, and Maximum Basophils (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

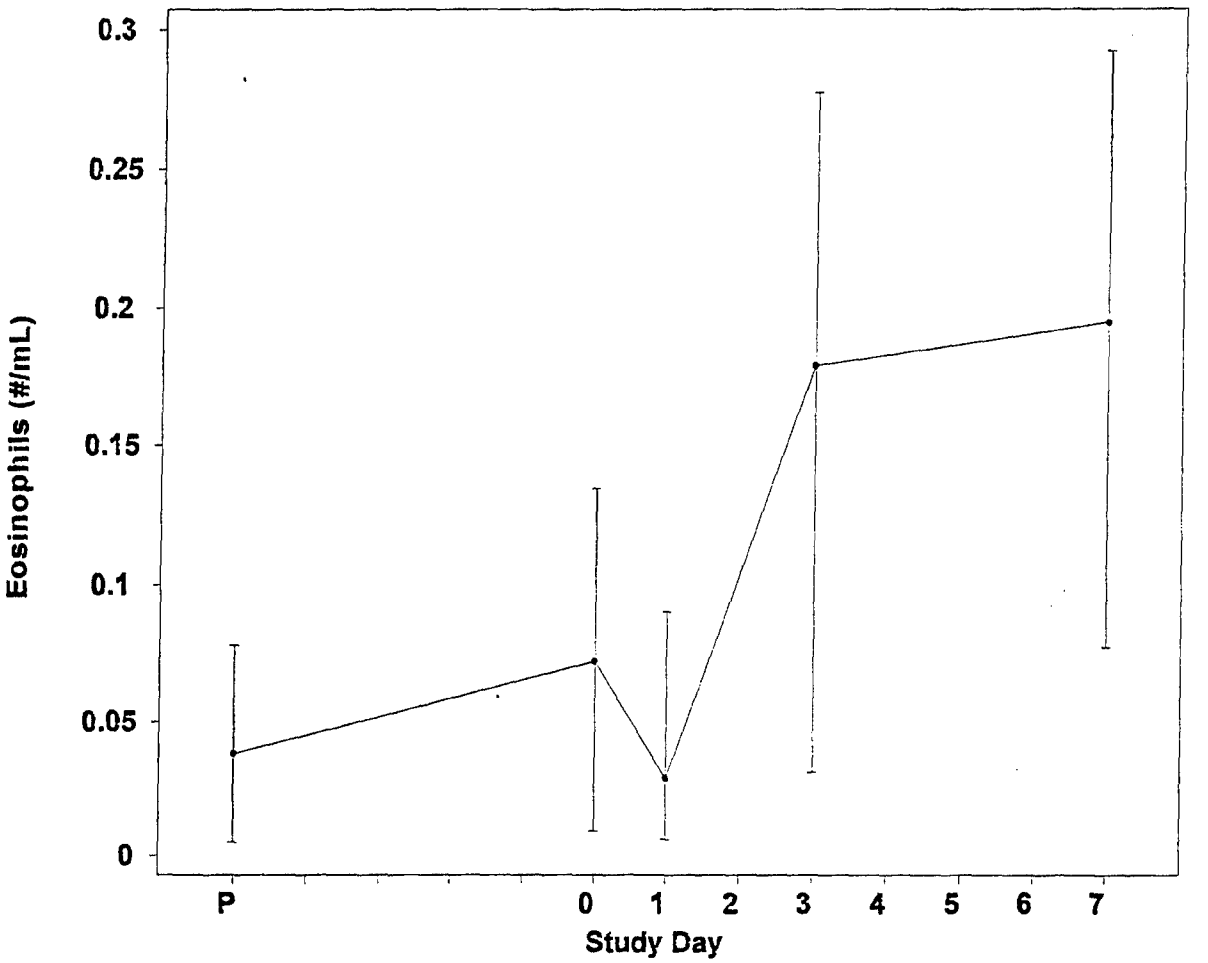

Figure 26. Mean, Minimum, and Maximum Eosinophils (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

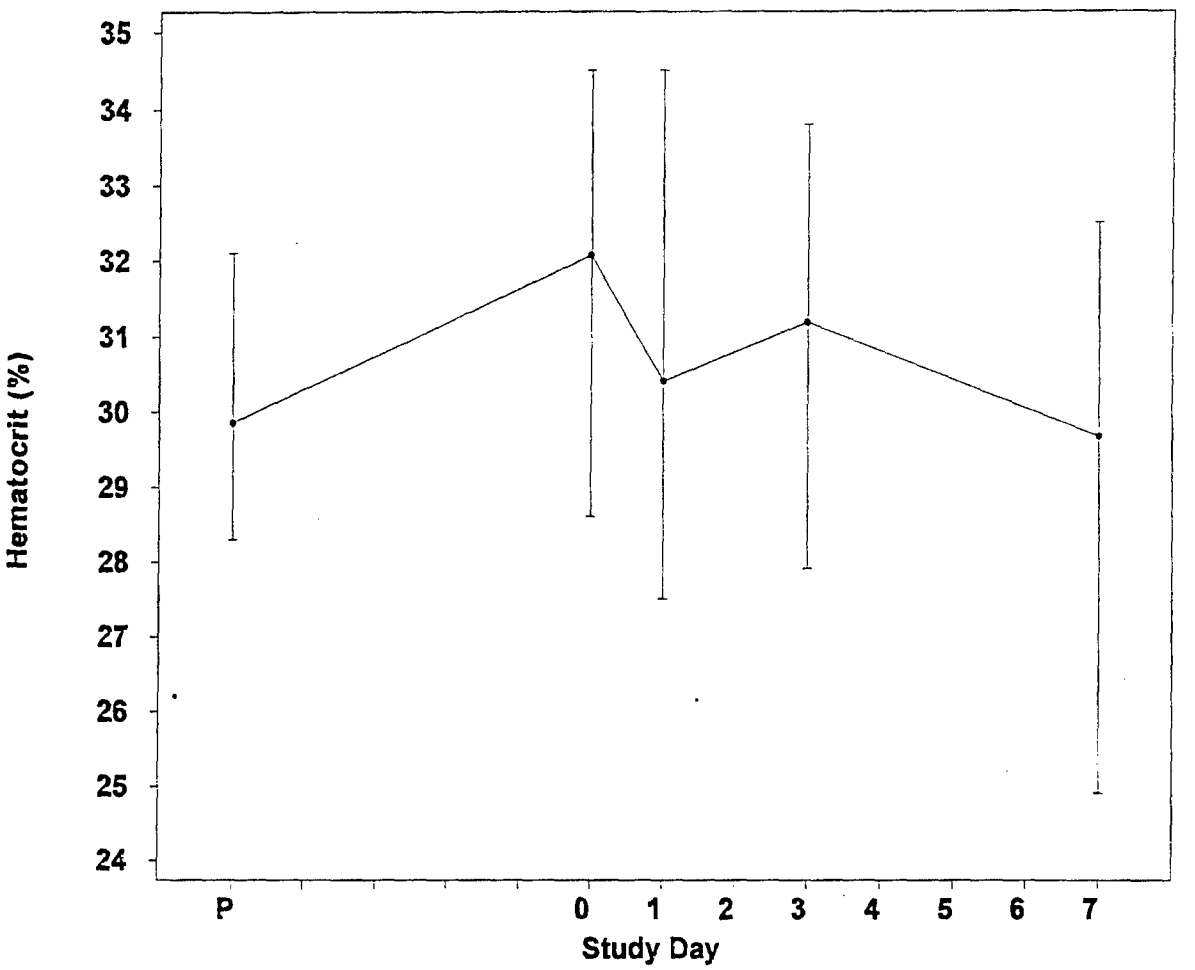

Figure 27. Mean, Minimum, and Maximum Hematocrit (%) by Study Day for the Six Animals Tested in Phase I, Part B.

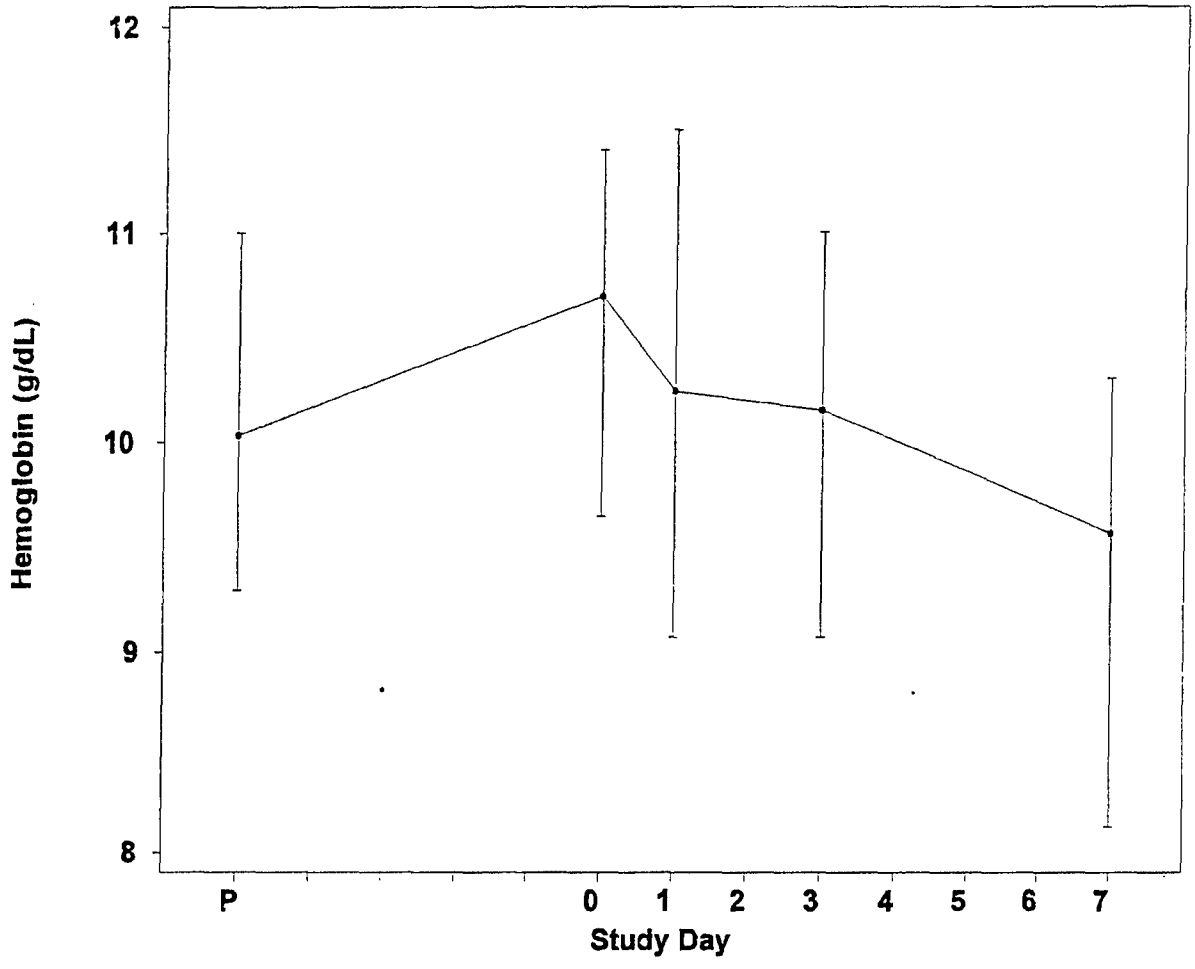

Figure 28. Mean, Minimum, and Maximum Hemoglobin (g/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

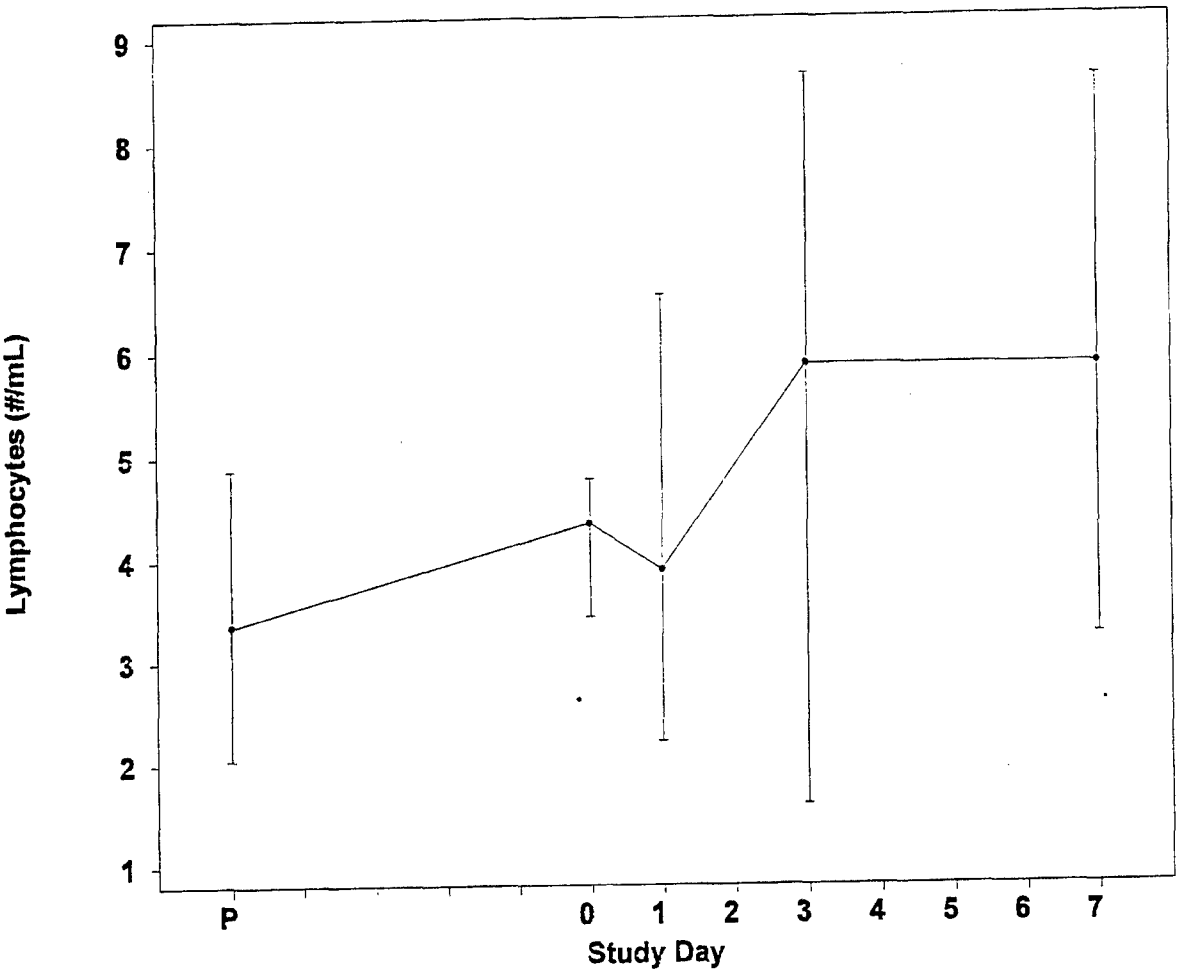

Figure 29. Mean, Minimum, and Maximum Lymphocytes (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

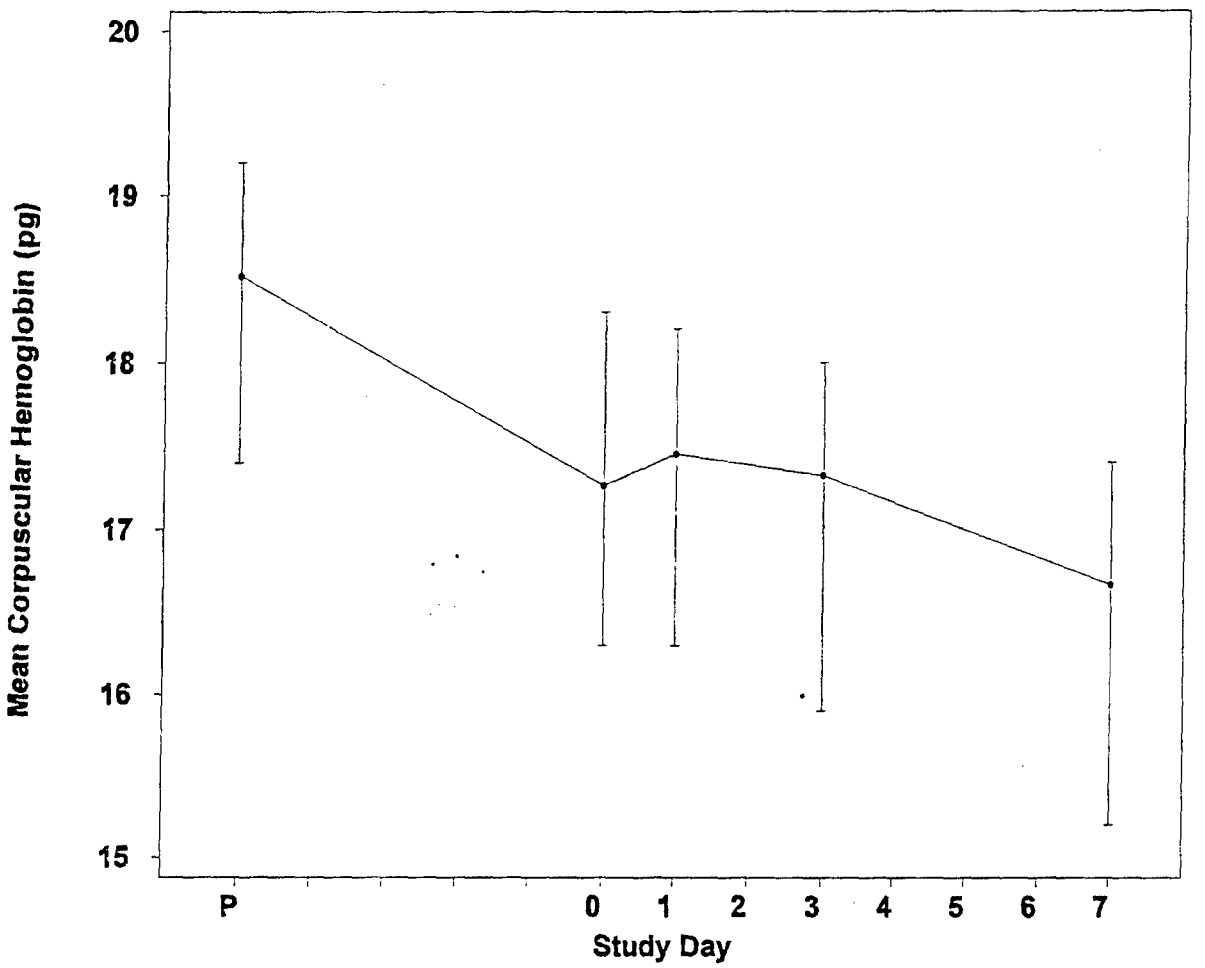

Figure 30. Mean, Minimum, and Maximum Mean Corpuscular Hemoglobin (pg) by Study Day for the Six Animals Tested in Phase I, Part B.

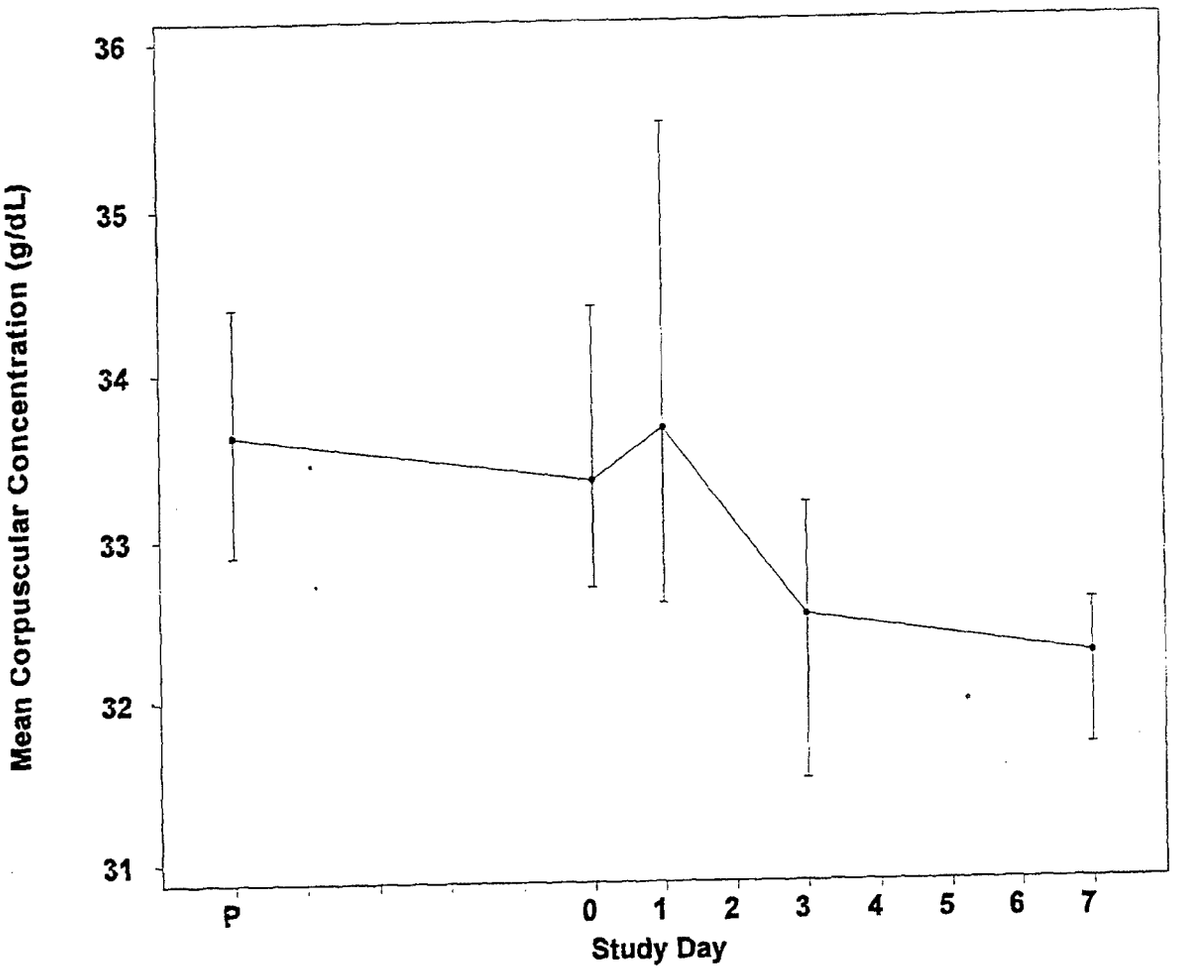

Figure 31. Mean, Minimum, and Maximum Mean Corpuscular Concentration (g/dL) by Study Day for the Six Animals Tested in Phase I, Part B.

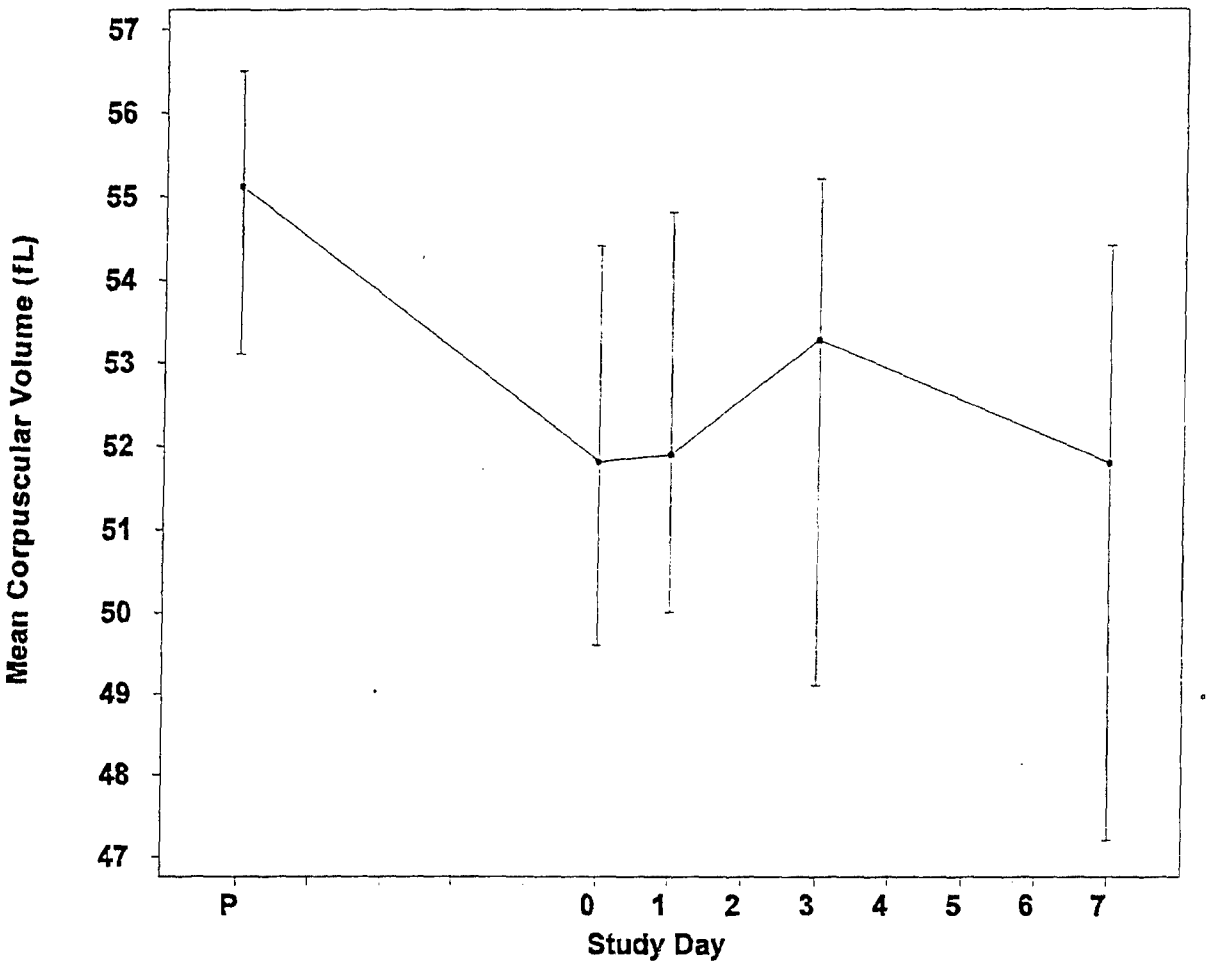

Figure 32. Mean, Minimum, and Maximum Mean Corpuscular Volume (fL) by Study Day for the Six Animals Tested in Phase I, Part B.

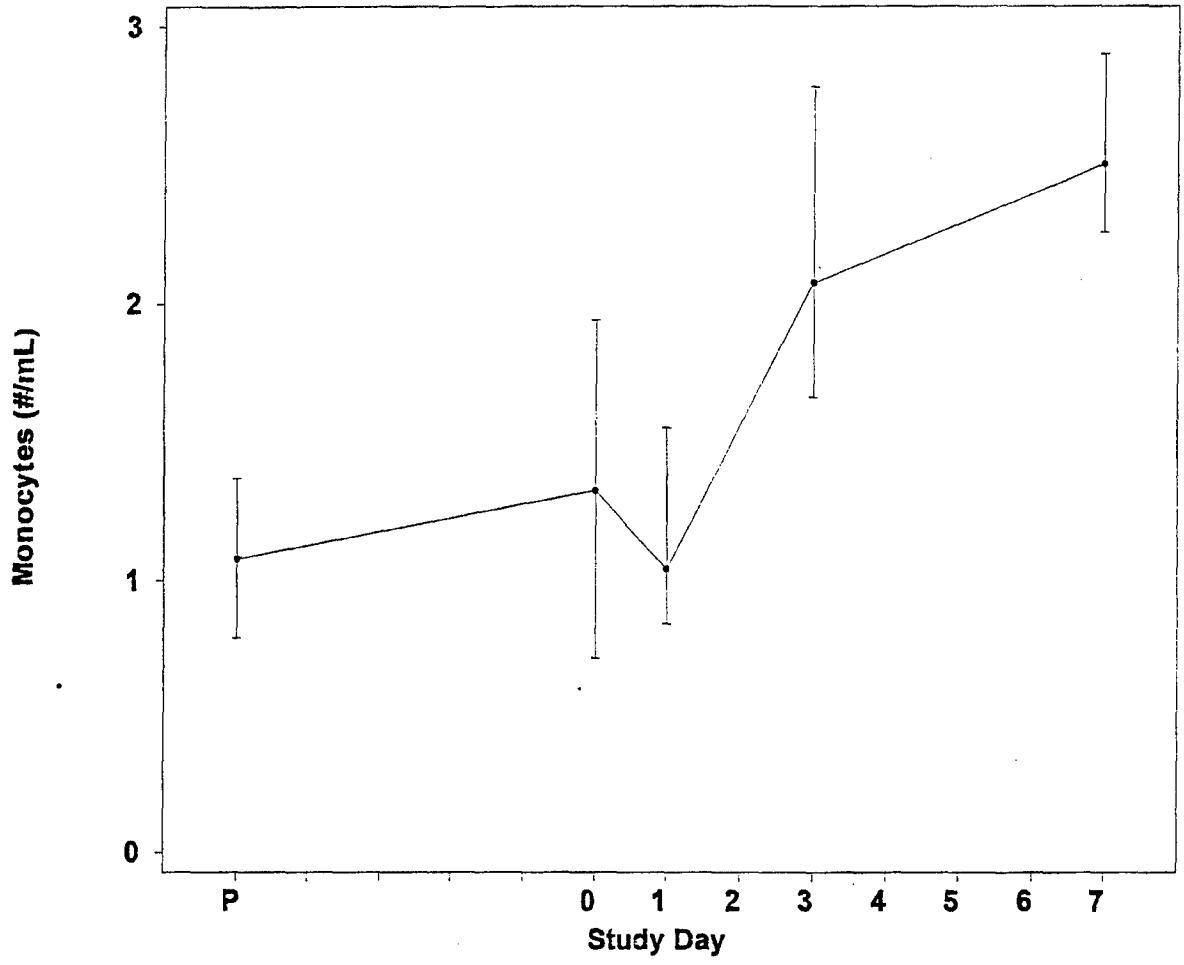

Figure 33. Mean, Minimum, and Maximum Monocytes (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

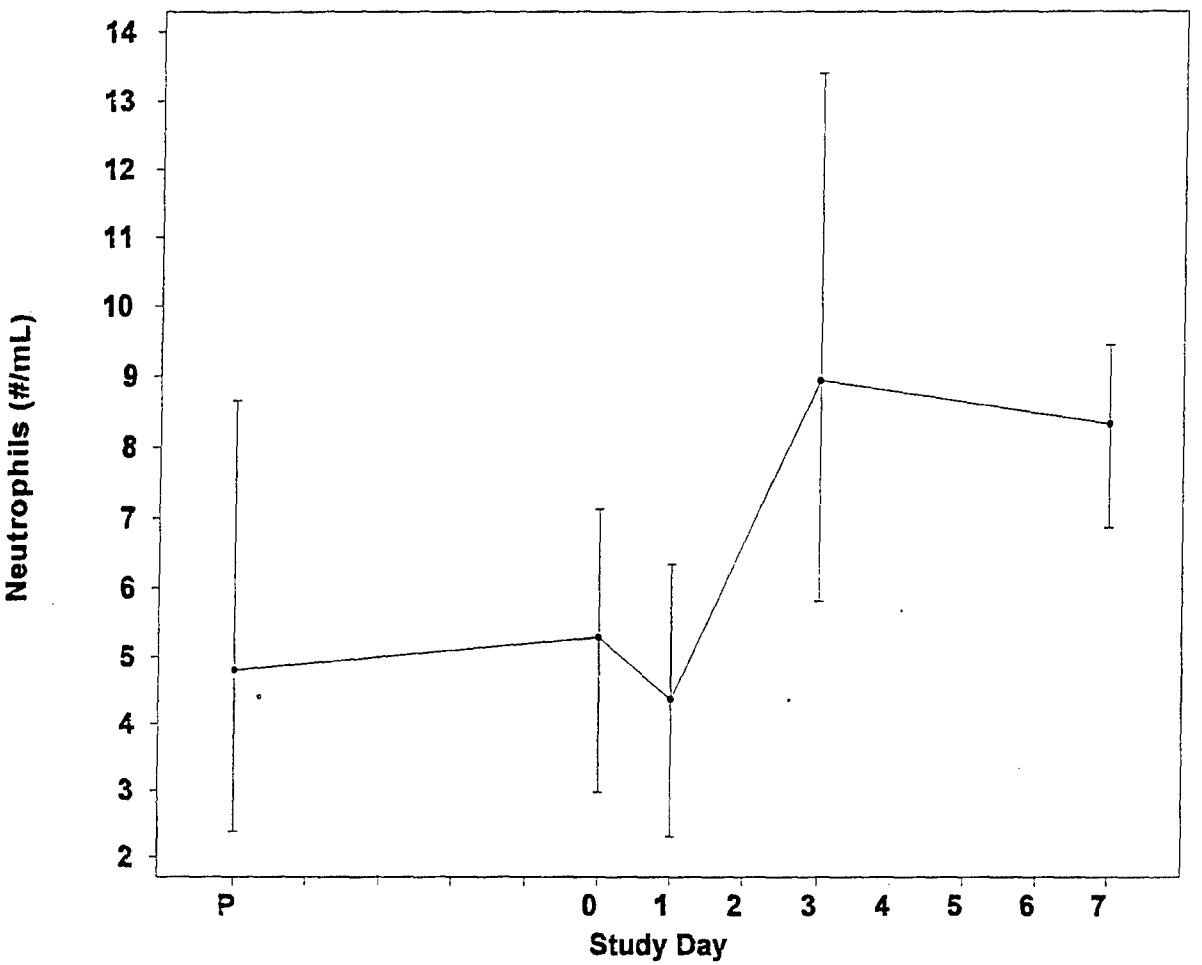

Figure 34. Mean, Minimum, and Maximum Neutrophils (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

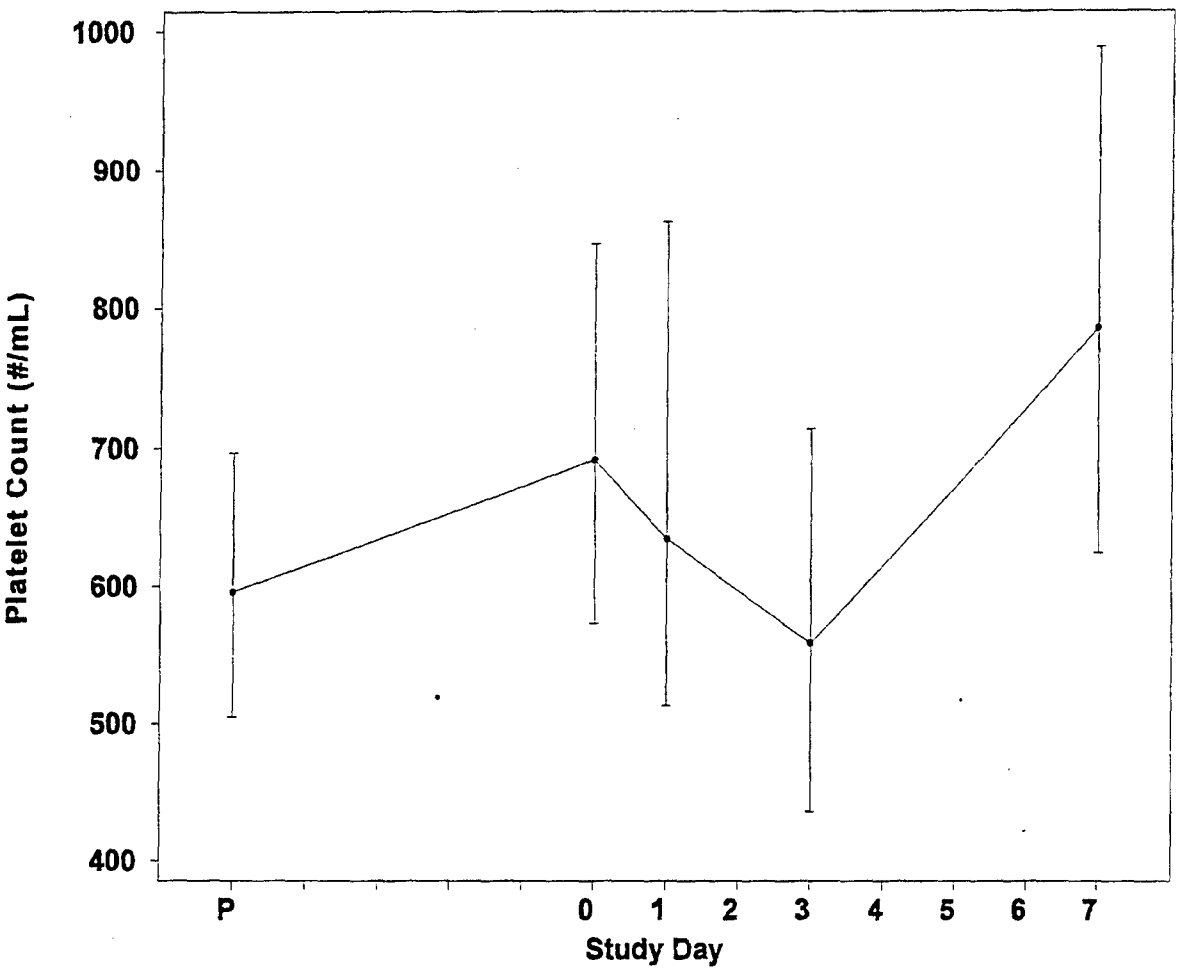

Figure 35. Mean, Minimum, and Maximum Platelet Count (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

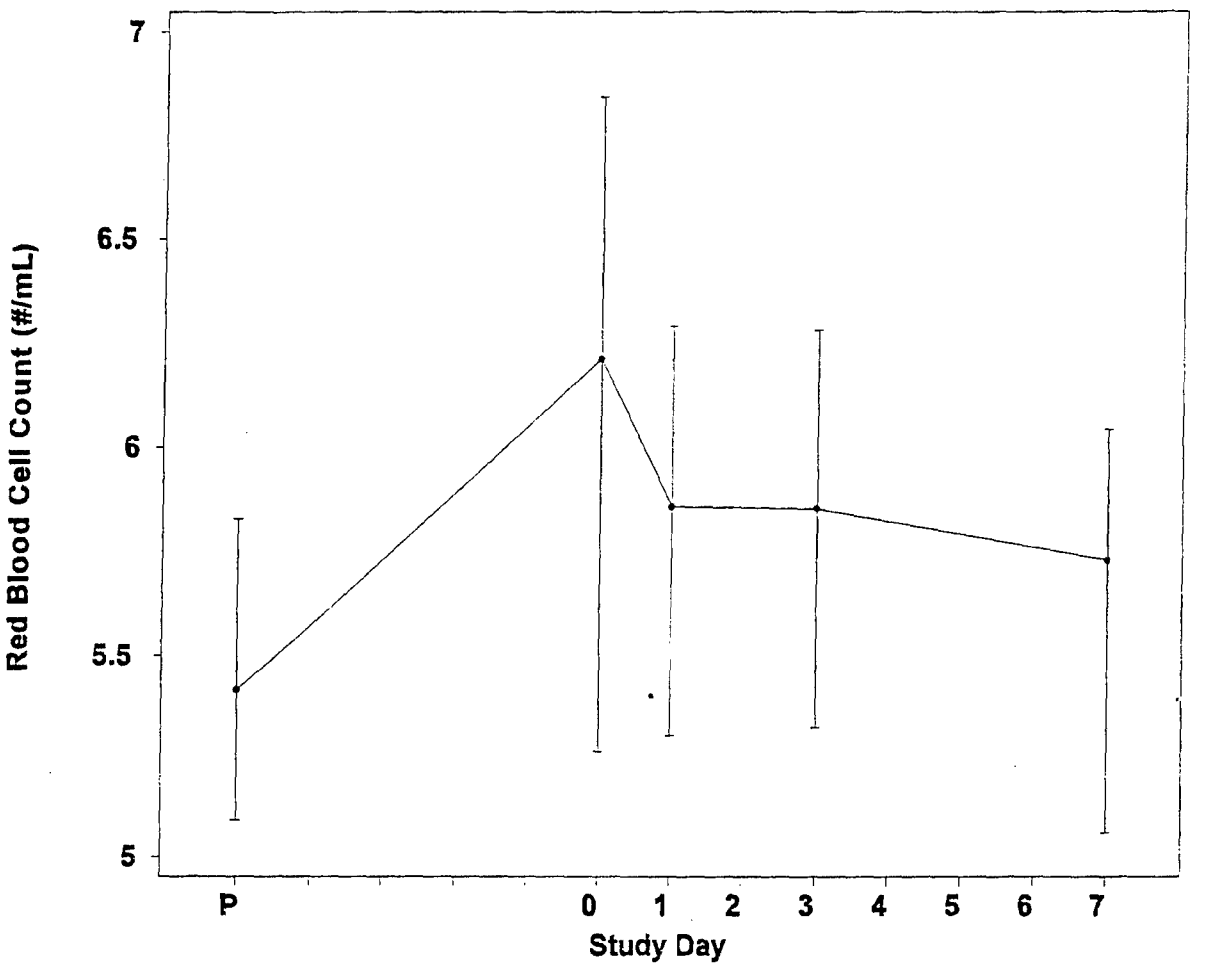

Figure 36. Mean, Minimum, and Maximum Red Blood Cell Count (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

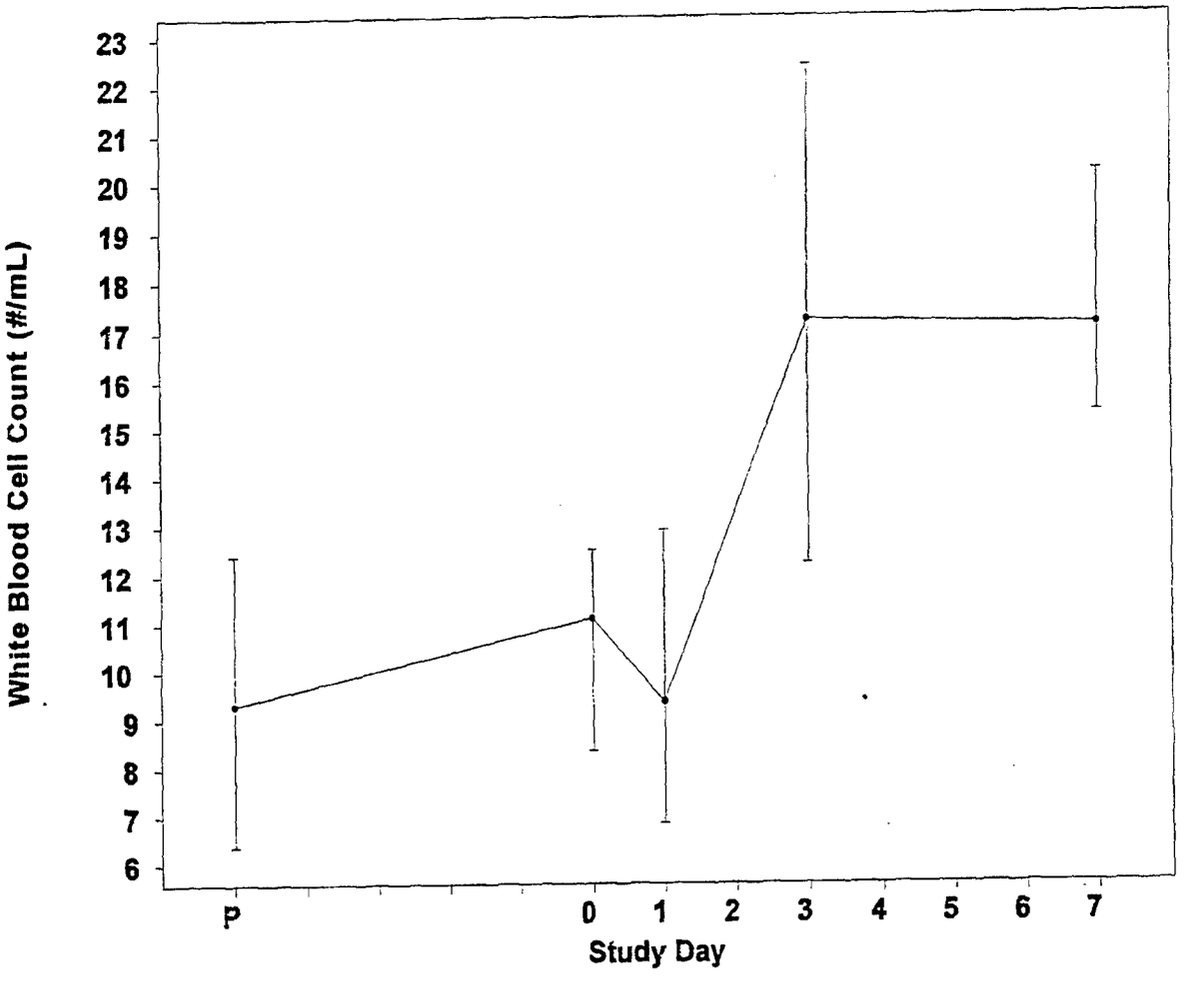

Figure 37. Mean, Minimum, and Maximum White Blood Cell Count (#/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

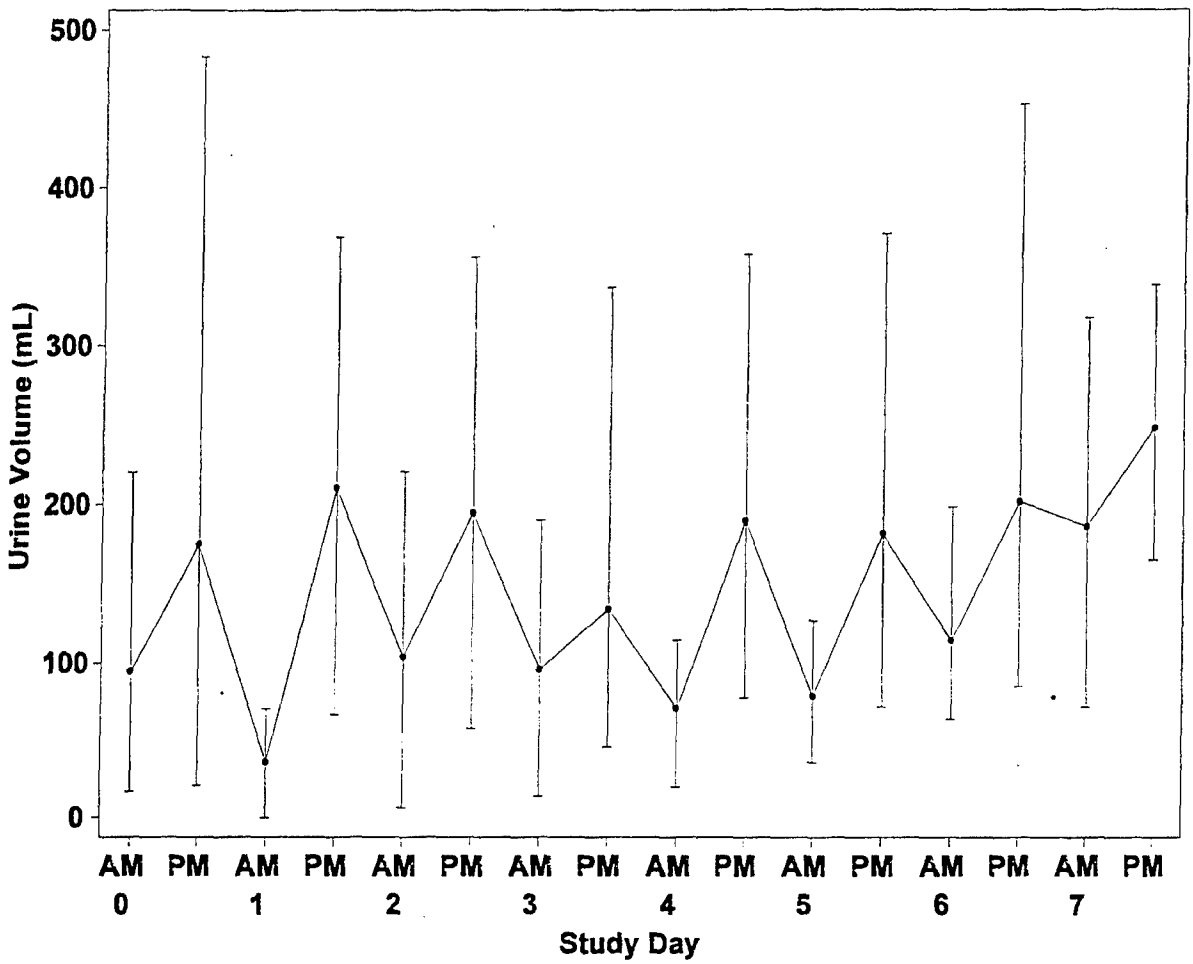

Figure 38. Mean, Minimum, and Maximum Urine Volume (mL) by Study Day for the Six Animals Tested in Phase I, Part B.

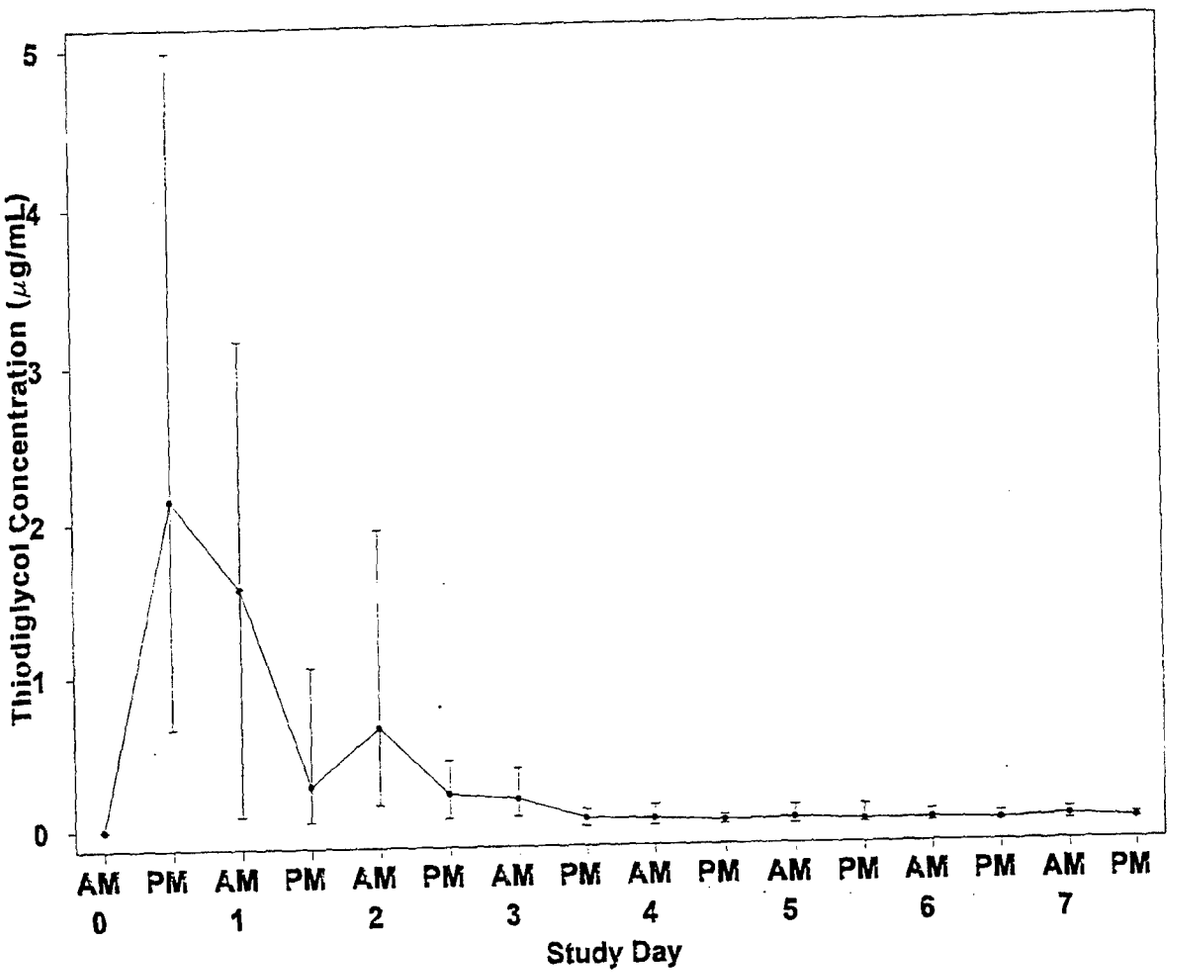

Figure 39. Mean, Minimum, and Maximum Thiodiglycol Concentration (µg/mL) by Study Day for the Six Animals Tested in Phase I, Part B.

ATTACHMENT E

Phase II Statistics Report

Project Number G1555-B33ASTAT

Battelle
. . . Putting Technology To Work

Internal Distribution

Department Files
N. Niemuth
S. Shumaker
J. Nagaraja
B. Skarpness
RMO

Date August 5, 1998

To Frances Reid

From Shawn M. Shumaker

Subject **Phase II of Task 94-33**

s:\shum\Task33\PhaseIIReportImemo.wpd
s:\shum\Task33\Phase2 Report 1.wpd

Attached is the statistical report on clinical observation and histopathological data collected in MREF Task 94-33, Phase II. A WordPerfect 8.0 file with the text, tables, and figures will be sent via e-mail for inclusion into your draft report.

SMS:llj
Attachment

For Review and Approval

	Name	Initials	Date
Originator	S. Shumaker	SMS	8/6/98
Concurrence	N. Niemuth	N	8/6/98
Approved	B. Skarpness	BS	8/6/98

MREF Task 94-33, Phase II

Statistical Report on Analysis of Histopathological Endpoints and Clinical Observations Data

Introduction

Twenty-four weanling pigs with six sites per animal were used to compare the efficacy of Dermagraft-TC temporary wound dressing (TWD), pig skin autograft, and no treatment in treating HD-induced dermal lesions. Two sites per animal were assigned to each treatment. Clinical evaluations of each wound were made approximately on day 2, 10, 17, 24, 31 and 38. The clinical observation parameters evaluated in this experiment were adherence, contraction/closure, durability, edema, epithelialization, erythema, eschar, exudate, granulation, inflammation, necrosis, rejection, wound size, and vascularization. Histological evaluations were done only on day 38. The histopathological indicators evaluated in this experiment were depth of necrosis, necrosis of basal epithelium, ulceration (loss of epidermis), granulation tissue response, and re-epithelialization (hyperplasia).

Statistical Methods

The primary objective of the statistical analysis of the histopathological endpoints and clinical observations parameters was to evaluate the efficacy of Dermagraft-TC TWD and pig skin autograft treatments compared to control (no treatment) and each other.

Wound Development

Some statistical comparison were based on the assumption that Phase II wounds were similar to Phase I, Part B wounds. Clinical observations parameters from Phase II and Phase I, Part B were used to determine if wounds from Phase II and Phase I, Part B were similar on day 2. Comparisons of clinical observation parameters between the two data sets were made using two sample t-tests (SAS PROC TTEST) and plots.

Histopathology

Analysis of variance (ANOVA) models were fitted to the depth of necrosis, granulation tissue response, and re-epithelialization day 38 data to determine the effect of wound treatment and to estimate animal-to-animal variation. Appropriate contrasts were used to assess whether there were differences between wound treatments. The SAS (V6.12) MIXED procedure was used to fit the ANOVA models. A logistic regression model was fitted to the ulceration data (scored as present/absent) using the SAS (V6.12) GENMOD procedure.

Since histopathology endpoints were observed only on day 38 in Phase II, data from Phase I, Part B on day 2 were combined with Phase II data to evaluate wound severity and degree of wound healing over time. The SAS (V6.12) MIXED procedure was used to fit ANOVA models to the combined data for each endpoint and appropriate contrasts were used to compare day 2 and day 38 histopathological endpoints.

Summary tables were prepared for histopathological indicators, which displayed number of observations, mean, standard deviation, minimum, maximum, and percent incidence. Mean scores for histopathological endpoints overlaid on observed values were plotted for each treatment group.

Clinical Observations

ANOVA models were fitted to clinical observations scores and included the effect of wound treatment, the effect of study days, an effect due to the interaction between wound treatment and study days, and a random animal effect. Pairwise comparisons between wound treatments were carried out using appropriate contrasts in the analysis of variance model (SAS PROC MIXED).

Data summary tables prepared for clinical observation parameters data displayed number of observations, mean, standard deviation, minimum and maximum. Mean scores and their associated 95% confidence intervals for each wound treatment group were also graphically displayed.

Results

Wound Development

Descriptive statistics and two sample t-test results used for comparing Phase II and Phase I Part B clinical signs on day 2, are presented in Table 1. Statistically significant differences between Phase I, Part B and Phase II were observed for erythema, edema, necrosis and wound size. Figures 1-5 illustrate the differences in the clinical observation scores between Phase II and Phase I, Part B. Since there appeared to be significant differences in wound development between Phase I, Part B and Phase II, the results of analyses combining data from both phases should be interpreted with caution.

Histopathology

Table 2 presents summary statistics for histopathological indicators on study day 38. As indicated in Table 2, necrosis of the basal epithelium was not present in any of the sites examined, for any treatment. Ulceration (scored as present/absent) was observed in 39% of no treatment sites, compared to 4% of autograft and 18% of Dermagraft-TC temporary wound dressing (TWD) sites. Re-epithelialization was nearly complete in autograft sites, where the mean score (3.96) was near maximum. Figures 6-9 present the mean depth of necrosis, ulceration, granulation tissue response, and re-epithelialization scores, respectively, overlaid on observed values for the three treatment groups.

Results of ANOVA models fitted to the depth of necrosis, granulation tissue response, and re-epithelialization scores on day 38 are presented in Table 3. No differences between treatment groups were noted in the analysis of depth of necrosis and granulation tissue response. Animal-to-animal variability was highly significant for these endpoints. Re-epithelialization scores were significantly greater in autograft sites, compared to no treatment and Dermagraft-TC TWD sites. The difference between no treatment and TWD was not statistically significant. Table 3 also provides the results of the logistic regression model fitted to the ulceration data. Ulceration was present in a significantly greater proportion of no treatment sites compared to

autograft and TWD sites. Also, ulceration was present in a significantly greater proportion of TWD sites compared to autograft sites. No statistical models were fitted to necrosis of basal epithelium as it was not present in any of the sites examined.

Results of the statistical comparisons of day 2 and day 38 histopathological endpoints for each wound treatment are displayed in Table 4. Granulation scores were significantly higher on day 38 than on day 2 for untreated, autograft and dermagraft sites. Depth of necrosis did not show any marked difference between day 2 and day 38 for any wound treatment. Ulceration was absent and epithelium was not lost for all animals on day 2 in Phase I, Part B, and therefore comparisons were not carried out for ulceration and re-epithelialization scores. Since most of the clinical observation parameters on study day 2 show statistically significant differences between Phase I, Part B and Phase II, the results of this analysis should be interpreted with caution.

Clinical Observations

Descriptive statistics for all clinical observations endpoints are presented in Table 5. From this table, it is evident that erythema, edema, and necrosis peaked early in the study for all treatment groups, whereas other clinical signs that are more indicative of healing, such as epithelialization, eschar, exudate, and granulation, peaked or were evident later in the study. Infection was rare and observed only in a small number of sites on days 10 and 17. Contraction scores appeared to be greater for no treatment sites. Adherence, durability, and rejection were not evaluated for the untreated sites nor for the wounds that were completely healed. Figures 10-23 present mean clinical observation scores plotted against time for each treatment, with 95% confidence intervals. It is evident from these figures that for most of the clinical observation parameters the wounds healed more rapidly in autograft sites, compared to the other treatments, and that TWD sites healed more rapidly than no treatment sites.

The results of the random effect model fitted to clinical observation parameters to assess animal-to-animal variability and the effect of wound treatment over time are summarized in Table 6. The overall study days effect and animal-to-animal effect were statistically significant for all clinical observation parameters. A significant difference due to wound treatment was observed for all parameters except erythema, exudate, granulation and infection. Wound severity scores (eschar, necrosis,and wound size) tended to be significantly lower for autograft sites than untreated and dermagraft sites. Wound healing scores (contraction, epithelialization, and vascularization) showed that wounds healed significantly faster for autograft sites than untreated sites and dermagraft sites and that dermagraft sites healed faster than untreated sites. Graft evaluation scores (adherence, durability, and rejection) were significantly lower for autograft sites than dermagraft sites. Significant interactions between study day and wound treatment effects indicate that the main trend varied over time. These effects are best seen in Figures 10-23. For example, adherence scores on study day 10 appeared to be greater for autograft than dermagraft sites, while the opposite was observed on later study days (Figure 10). The interaction effect was not significant for contraction and, a seen in Figure 11, the relationship between treatment scores is consistent over time.

Conclusions

Overall, pig skin autograft sites healed faster and wounds at 38 days were less severe compared to Dermagraft-TC and no treatment sites. In addition, Dermagraft -TC TWD sites healed more rapidly than no treatment sites. Based on the analysis of clinical signs, Phase I, Part

Table 1. Two-Sample T-Test Results for Comparing Phase II and Phase I Part B Clinical Observation Parameters on Study Day2.

Parameter	N		Mean		Std. Deviation		P-Value
	Phase I Part B	Phase II	Phase I Part B	Phase II	Phase I Part B	Phase II	
Exudate	36	144	0.30	0.25	0.66	0.87	NS
Erythema	36	144	4.68	3.35	0.37	0.51	0.0001
Edema	36	144	4.47	2.19	0.87	0.97	0.0001
Necrosis	36	144	1.23	1.56	0.49	1.27	0.0149
Wound Size	36	144	1565.2	1397.5	341.18	178.45	0.0068

NS = Not Significant at the 0.05 level of significance.

B and Phase II wounds developed differently to day 2, thus the interpretation of comparisons of histopathologic endpoints between day 2 (Phase I, Part B) and day 38 (Phase II) is not clear.

Table 2. Descriptive Statistics of Histopathologic Endpoints, on Study Day 38

Histopathology Endpoints	Treatment Groups	N	Mean	Std. Deviation	Minimum	Maximum	Percent Incidence (%)
Depth of Necrosis	No Treatment	47	3.72	0.58	2	4	100
	Pig Skin Autograft	48	3.67	0.56	2	4	100
	Dermagraft - TC TWD	48	3.77	0.47	2	4	100
Necrosis of Basal Epithelium	No Treatment	47	0.00	0.00	0	0	0
	Pig Skin Autograft	48	0.00	0.00	0	0	0
	Dermagraft - TC TWD	48	0.00	0.00	0	0	0
Ulceration	No Treatment	46	0.39	0.49	0	1	39
	Pig Skin Auto	48	0.04	0.20	0	1	4
	Dermagraft - TC TWD	44	0.18	0.39	0	1	18
Granulation Tissue Response	No Treatment	47	3.83	0.48	2	4	100
	Pig Skin Autograft	48	3.71	0.62	1	4	100
	Dermagraft - TC TWD	48	3.83	0.43	2	4	100
Re-epitheliazation	No Treatment	47	3.68	0.52	2	4	100
	Pig Skin Autograft	48	3.96	0.20	3	4	100
	Dermagraft - TC TWD	46	3.72	0.58	2	4	100

Table 3. ANOVA and Logistic Regression Results for Histopathological Endpoints on Study Day 38.

Parameter	Overall P-Value for Treatment Group Differences	P-Values for Treatment Group Comparisons			P-Value for Animal-to-Animal Variability
		NoTreatment vs Autograft (Mean±SE)	No Treatment vs Dermagraft (Mean±SE)	Autograft vs Dermagraft (Mean±SE)	
Depth of Necrosis[1]	NS	NS	NS	NS	0.0001
Granulation Tissue Response[1]	NS	NS	NS	NS	0.0001
Re-epithelialization[1]	0.0067	0.0035 (-0.28±0.09)	NS	0.0116 (0.24±0.09)	NS
Ulceration[2]	0.0001	0.0001	0.0268	0.0268	-

1. ANOVA results are presented for this parameter.
2. Logistic regression results are presented for this parameter.

NS = Not significant at the 0.05 level of significance.

Table 4. Results of Comparisons of Day 2 (Phase I, Part B) and Day 38 (Phase II) Histopathological Endpoints.

Parameter	P-Value for Day 2 and Day 38 Comparisons		
	No Treatment (Mean±SE)	Autograft (Mean±SE)	Dermagraft (Mean±SE)
Depth of Necrosis	NS	NS	NS
Granulation Tissue Response	0.0001 (3.8±0.16)	0.0001 (3.7±0.16)	0.0001 (3.8±0.16)

NS = Not significant at the 0.05 level of significance.

Table 5. Descriptive Statistics of Clinical Observations Parameters, on Study Days 2, 10, 17, 24, 31, and 38.

Parameters	Study Day	No Treatment					Pig Skin Autograft					Deramagraft - TC TWD				
		N	Mean	Std	Min	Max	N	Mean	Std	Min	Max	N	Mean	Std	Min	Max
Adherence [1]	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	0	.	.	.	.	48	2.02	1.19	0.00	4.00	48	1.15	1.28	0.00	4.00
	17	0	.	.	.	.	47	2.14	0.77	0.75	3.00	48	2.85	1.74	0.00	4.00
	24	0	.	.	.	.	47	1.84	0.91	0.25	4.00	48	3.57	1.14	0.00	4.00
	31	0	.	.	.	.	42	1.22	0.77	0.00	3.00	48	4.00	0.00	4.00	4.00
	38	0	.	.	.	.	16	1.75	1.18	0.00	4.00	38	3.84	0.68	1.00	4.00
Contraction	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	48	2.86	1.70	0.00	4.00	48	2.64	1.62	0.00	4.00	43	2.73	1.74	0.00	4.00
	17	48	3.34	0.81	0.00	4.00	48	2.60	1.12	0.00	4.00	43	2.84	0.70	1.00	4.00
	24	48	2.79	0.92	0.00	4.00	48	2.09	1.30	0.00	4.00	48	2.16	0.84	0.00	3.50
	31	48	1.45	0.85	0.00	3.00	48	1.14	1.16	0.00	4.00	48	1.35	0.73	0.00	3.00
	38	47	0.88	0.92	0.00	3.00	48	0.35	0.85	0.00	3.00	48	1.04	0.96	0.00	3.00
Durability [1]	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	0	.	.	.	.	48	2.09	1.18	0.00	4.00	48	0.60	1.16	0.00	4.00
	17	0	.	.	.	.	47	2.11	0.78	0.75	3.25	44	2.61	1.91	0.00	4.00
	24	0	.	.	.	.	47	1.88	0.88	0.00	4.00	46	3.54	1.13	0.00	4.00
	31	0	.	.	.	.	42	1.20	0.81	0.00	3.00	48	4.00	0.00	4.00	4.00
	38	0	.	.	.	.	16	1.67	1.25	0.00	4.00	38	3.84	0.68	1.00	4.00

Table 5. Descriptive Statistics of Clinical Observations Parameters, on Study Days 2, 10, 17, 24, 31, and 38 (Continued).

Parameters	Study Day	No Treatment					Pig Skin Autograft					Deramagraft - TC TWD				
		N	Mean	Std	Min	Max	N	Mean	Std	Min	Max	N	Mean	Std	Min	Max
Edema	2	48	2.10	0.99	0.00	4.00	48	2.33	0.99	1.00	4.00	48	2.14	0.93	0.00	4.00
	10	48	0.89	0.97	0.00	4.00	48	0.24	0.43	0.00	1.00	46	0.17	0.57	0.00	3.00
	17	48	0.00	0.00	0.00	0.00	48	0.01	0.07	0.00	0.50	46	0.00	0.00	0.00	0.00
	24	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
	31	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
	38	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
Epithelialization	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	48	3.36	1.32	0.00	4.00	48	2.46	1.14	0.00	4.00	33	3.06	1.51	0.00	4.00
	17	42	3.53	0.65	1.00	4.00	48	2.05	0.83	0.00	3.25	38	2.78	0.86	0.50	4.00
	24	45	2.83	0.81	1.00	4.00	45	1.55	0.69	0.00	3.00	43	2.15	0.86	0.00	3.50
	31	48	1.09	0.65	0.00	3.00	48	0.60	0.42	0.00	1.00	48	0.98	0.53	0.00	2.25
	38	47	0.54	0.51	0.00	2.00	48	0.15	0.32	0.00	1.00	48	0.52	0.57	0.00	3.00
Erythema	2	48	3.34	0.50	2.00	4.00	48	3.36	0.52	2.00	4.00	48	3.35	0.52	2.00	4.00
	10	48	1.20	0.92	0.00	4.00	48	1.40	1.38	0.00	4.00	35	0.97	1.03	0.00	4.00
	17	48	0.58	1.33	0.00	4.00	48	0.54	1.34	0.00	4.00	45	0.66	1.38	0.00	4.00
	24	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
	31	48	0.02	0.14	0.00	1.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
	38	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00

Table 5. Descriptive Statistics of Clinical Observations Parameters, on Study Days 2, 10, 17, 24, 31, and 38 (Continued).

Parameters	Study Day	No Treatment					Pig Skin Autograft					Deramagraft - TC TWD				
		N	Mean	Std	Min	Max	N	Mean	Std	Min	Max	N	Mean	Std	Min	Max
Eschar	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	48	1.28	1.81	0.00	4.25	48	0.99	1.12	0.00	3.50	41	0.84	1.38	0.00	3.50
	17	48	3.94	0.22	3.00	4.00	48	2.53	0.96	0.00	4.00	42	3.14	0.77	1.00	4.00
	24	48	2.86	1.16	1.00	4.00	48	1.86	1.03	0.00	4.00	48	2.04	1.09	0.00	4.00
	31	48	1.09	0.82	0.00	4.00	48	0.64	0.54	0.00	2.00	48	0.90	0.52	0.00	2.00
	38	48	0.43	0.44	0.00	1.00	48	0.12	0.26	0.00	1.00	48	0.43	0.55	0.00	3.00
Exudate	2	48	0.21	0.82	0.00	4.00	48	0.18	0.73	0.00	4.00	48	0.35	1.06	0.00	4.00
	10	48	0.88	0.65	0.00	3.50	48	0.95	0.48	0.00	2.00	47	1.20	0.71	0.00	4.00
	17	48	0.06	0.24	0.00	1.00	48	0.21	0.41	0.00	1.00	44	0.16	0.37	0.00	1.00
	24	46	0.34	0.52	0.00	2.00	48	0.21	0.46	0.00	2.00	47	0.18	0.38	0.00	1.00
	31	48	0.31	0.77	0.00	4.00	48	0.06	0.23	0.00	1.00	48	0.26	0.63	0.00	3.00
	38	48	0.13	0.61	0.00	4.00	48	0.00	0.00	0.00	0.00	48	0.15	0.65	0.00	4.00
Granulation	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	46	0.55	1.14	0.00	4.00	46	0.78	1.31	0.00	4.00	37	0.51	1.26	0.00	4.00
	17	28	1.29	1.78	0.00	4.00	35	1.19	1.27	0.00	4.00	27	1.41	1.64	0.00	4.00
	24	34	2.25	1.00	0.00	4.00	35	2.29	1.02	0.00	4.00	34	2.52	0.60	2.00	4.00
	31	45	1.87	1.04	0.00	4.00	46	1.77	1.32	0.00	4.00	44	1.80	0.99	0.00	4.00
	38	48	2.15	1.55	0.00	4.00	48	2.92	1.58	0.00	4.00	48	2.13	1.46	0.00	4.00

Table 5. Descriptive Statistics of Clinical Observations Parameters, on Study Days 2, 10, 17, 24, 31, and 38 (Continued).

Parameters	Study Day	No Treatment					Pig Skin Autograft					Deramagraft - TC TWD				
		N	Mean	Std	Min	Max	N	Mean	Std	Min	Max	N	Mean	Std	Min	Max
Infection	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	48	0.29	0.46	0.00	1.00	48	0.17	0.38	0.00	1.00	48	0.21	0.41	0.00	1.00
	17	48	0.00	0.00	0.00	0.00	48	0.04	0.20	0.00	1.00	48	0.02	0.14	0.00	1.00
	24	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
	31	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
	38	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
Necrosis	2	48	1.54	1.30	0.00	4.00	48	1.65	1.21	0.00	4.00	48	1.48	1.31	0.00	4.00
	10	48	3.80	0.53	2.00	4.25	48	2.60	1.20	0.00	4.00	31	3.14	1.18	0.00	4.00
	17	48	1.58	1.91	0.00	4.00	48	1.03	1.36	0.00	4.00	46	1.27	1.60	0.00	4.00
	24	48	0.52	1.27	0.00	4.00	48	0.49	1.17	0.00	4.00	48	0.36	0.89	0.00	4.00
	31	48	0.06	0.29	0.00	1.75	48	0.04	0.16	0.00	0.75	48	0.08	0.32	0.00	1.75
	38	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
Rejection [1]	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	0	.	.	.	.	48	2.15	1.22	0.00	4.00	48	1.06	1.21	0.00	4.00
	17	0	.	.	.	.	47	2.14	0.80	1.00	3.00	47	2.96	1.72	0.00	4.00
	24	0	.	.	.	.	47	1.80	0.88	0.25	4.00	48	3.50	1.25	0.00	4.00
	31	0	.	.	.	.	42	1.40	0.82	0.00	3.00	48	4.00	0.00	4.00	4.00
	38	0	.	.	.	.	16	1.81	1.22	0.00	4.00	38	3.84	0.68	1.00	4.00

Table 5. Descriptive Statistics of Clinical Observations Parameters, on Study Days 2, 10, 17, 24, 31, and 38 (Continued).

Parameters	Study Day	No Treatment					Pig Skin Autograft					Deramagraft - TC TWD				
		N	Mean	Std	Min	Max	N	Mean	Std	Min	Max	N	Mean	Std	Min	Max
Vascularization	2	0	.	.	.	.	0	.	.	.	.	0	.	.	.	.
	10	48	3.02	1.56	0.00	4.00	48	2.23	1.21	0.00	4.00	33	2.56	1.54	0.00	4.00
	17	38	3.08	0.96	0.25	4.00	48	1.80	0.88	0.00	3.00	36	2.32	0.82	1.00	3.25
	24	40	2.02	1.10	0.00	4.00	44	1.29	0.93	0.00	3.00	43	1.51	0.99	0.00	3.00
	31	48	0.36	0.73	0.00	3.00	48	0.11	0.31	0.00	1.00	48	0.34	0.57	0.00	2.00
	38	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00	48	0.00	0.00	0.00	0.00
Wound Size	2	48	1417	198	1072	1868	48	1414	168	1074	1809	48	1362	166	1100	1728
	10	48	1362	207	1005	1993	48	1180	426	346	2419	48	1377	251	895	1964
	17	48	846	131	589	1162	48	568	229	0	1056	48	750	192	393	1279
	24	48	543	185	212	895	48	347	210	0	1164	48	427	211	0	825
	31	48	162	132	0	615	48	57	53	0	242	48	124	93	0	434
	38	48	56	109	0	676	46	8	19	0	110	48	44	87	0	495

(1) Not evaluated for the non-treated sites. Also wounds that were completely healed were not evaluated.

Table 6. Statistical Results for Clinical Observations Parameters

Parameter	Fixed Effects			Random Effect	Pairwise Comparisons		
	Study Days	Wound Treatment	Interaction Between Study days and Wound Treatment	Animal-to-Animal Effect	Non Treated vs Autograft P-Values (Mean ± SE)[1]	Non Treated vs Dermagraft (Mean ± SE)[2]	Autograft vs Dermagraft (Mean ± SE)[3]
Adherence	0.0001	0.0001	0.0001	0.0001	-	-	0.0001 (-1.307 ± 0.102)
Contraction	0.0001	0.0001	NS	0.0001	0.0001 (0.499 ± 0.095)	0.0147 (0.236 ± 0.097)	0.0066 (-0.263 ± 0.096)
Durability	0.0001	0.0001	0.0001	0.0001	-	-	0.0001 (-1.144 ± 0.104)
Edema	0.0001	0.0171	0.0001	0.0002	NS	0.0046 (0.114 ± 0.04)	NS
Epithelialization	0.0001	0.0001	0.0001	0.0001	0.0001 (0.908 ± 0.072)	0.0001 (0.370 ± 0.074)	0.0001 (-0.537 ± 0.074)
Erythema	0.0001	NS	NS	0.0001	NS	NS	NS
Eschar	0.0001	0.0001	0.0001	0.0001	0.0001 (0.691 ± 0.082)	0.0001 (0.46 ± 0.083)	0.0057 (-0.231 ± 0.083)
Exudate	0.0001	NS	NS	0.0074	NS	NS	0.0214 (-0.113 ± 0.049)
Granulation	0.0001	NS	NS	0.0012	NS	NS	NS
Infection	0.0001	NS	NS	0.0001	NS	NS	NS

Table 6. Statistical Results for Clinical Observations Parameters (Continued)

Parameter	Fixed Effects			Random Effect	Pairwise Comparisons		
	Study Days	Wound Treatment	Interaction Between Study days and Wound Treatment	Animal-to-Animal Effect	Non Treated vs Autograft P-Values (Mean ± SE)[1]	Non Treated vs Dermagraft P-Values (Mean ± SE)[2]	Autograft vs Dermagraft P-Values (Mean ± SE)[3]
Necrosis	0.0001	0.0016	0.0003	0.0001	0.0005 (0.284 ± 0.086)	0.0126 (0.209 ± 0.084)	NS
Rejection	0.0001	0.0001	0.0001	0.0001	-	-	0.0001 (-1.236 ± 0.103)
Vascularization	0.0001	0.0001	0.0001	0.0001	0.0001 (0.610 ±0.081)	0.0001 (0.352 ± 0.084)	0.0019 (-0.259 ± 0.083)
Wound Size	0.0001	0.0001	0.0001	0.0001	0.0001 (135.305 ±14.751)	0.0006 (50.492 ± 14.723)	0.0001 (-84.814 ± 14.751)

1. Mean and standard error (SE) of the difference between non-treated and autograft sites averaged over the study days.
2. Mean and standard error (SE) of the difference between non-treated and dermagraft sites averaged over the study days.
3. Mean and standard error (SE) of the difference between non-treated and autograft and dermagraft sites averaged over the study days.

NS = Not significant at the 0.05 level of significance.

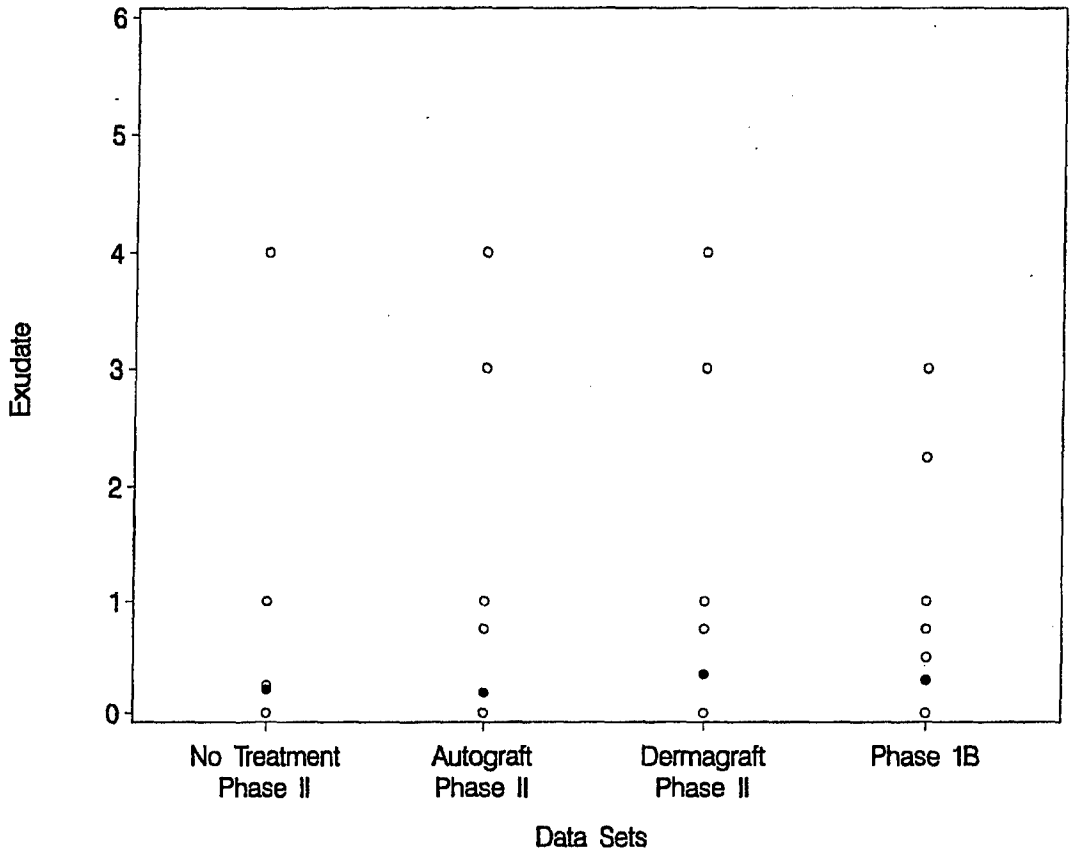

Figure 1. **Clinical Observation Exudate on Study Day 2 in Phase II and Phase I, Part B. Mean Exudate (.) Overlaid on Observed Values**

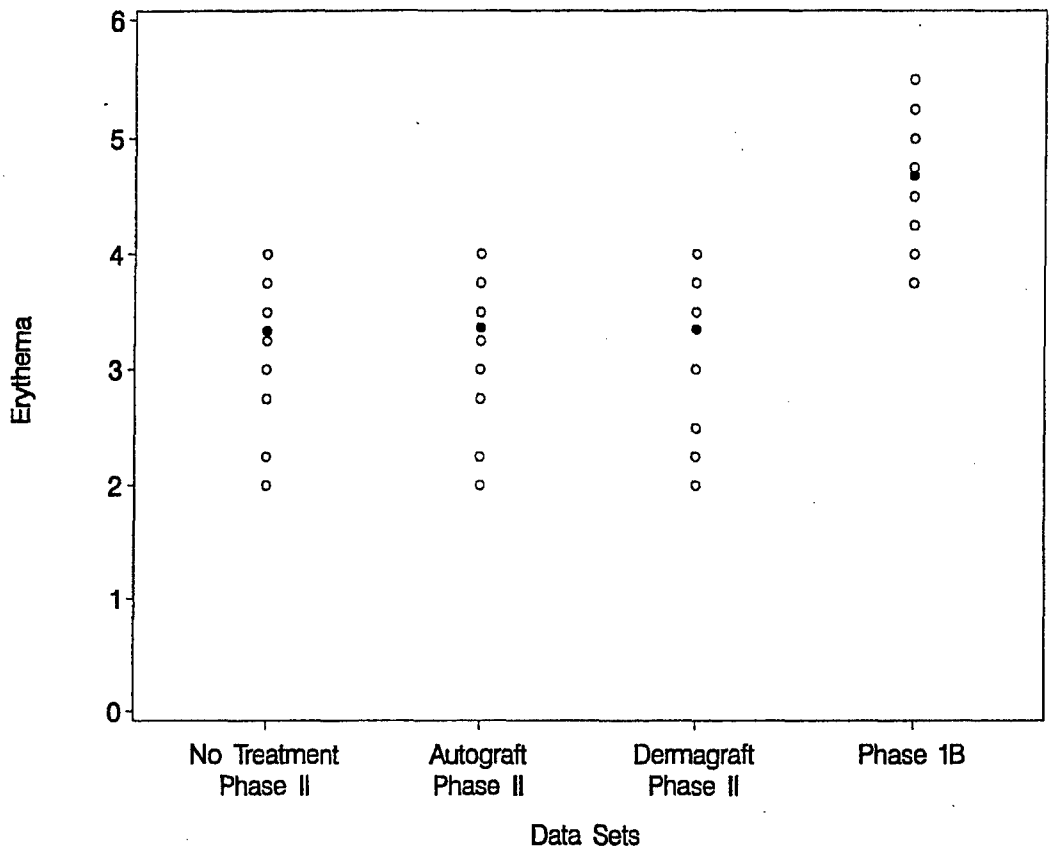

Figure 2. **Clinical Observation Erythema on Study Day 2 in Phase II and Phase I, Part B. Mean Erythema (.) Overlaid on Observed Values**

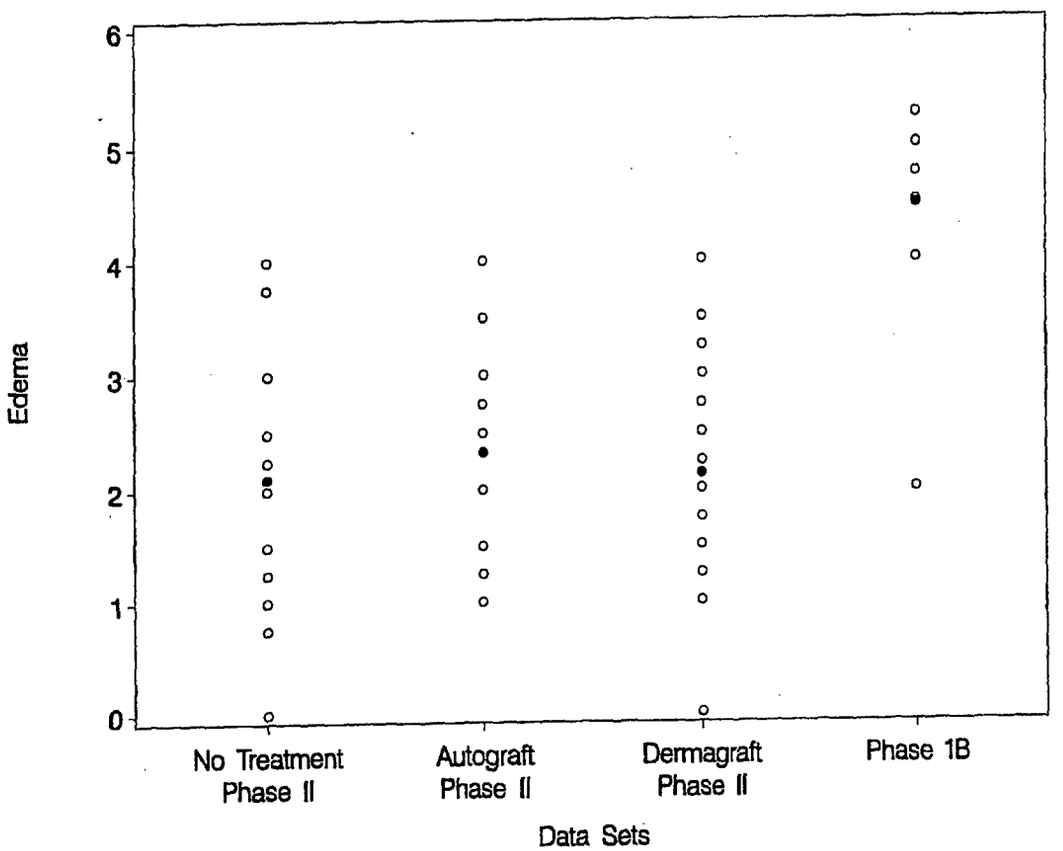

Figure 3. **Clinical Observation Edema on Study Day 2 in Phase II and Phase I, Part B. Mean Edema (.) Overlaid on Observed Values**

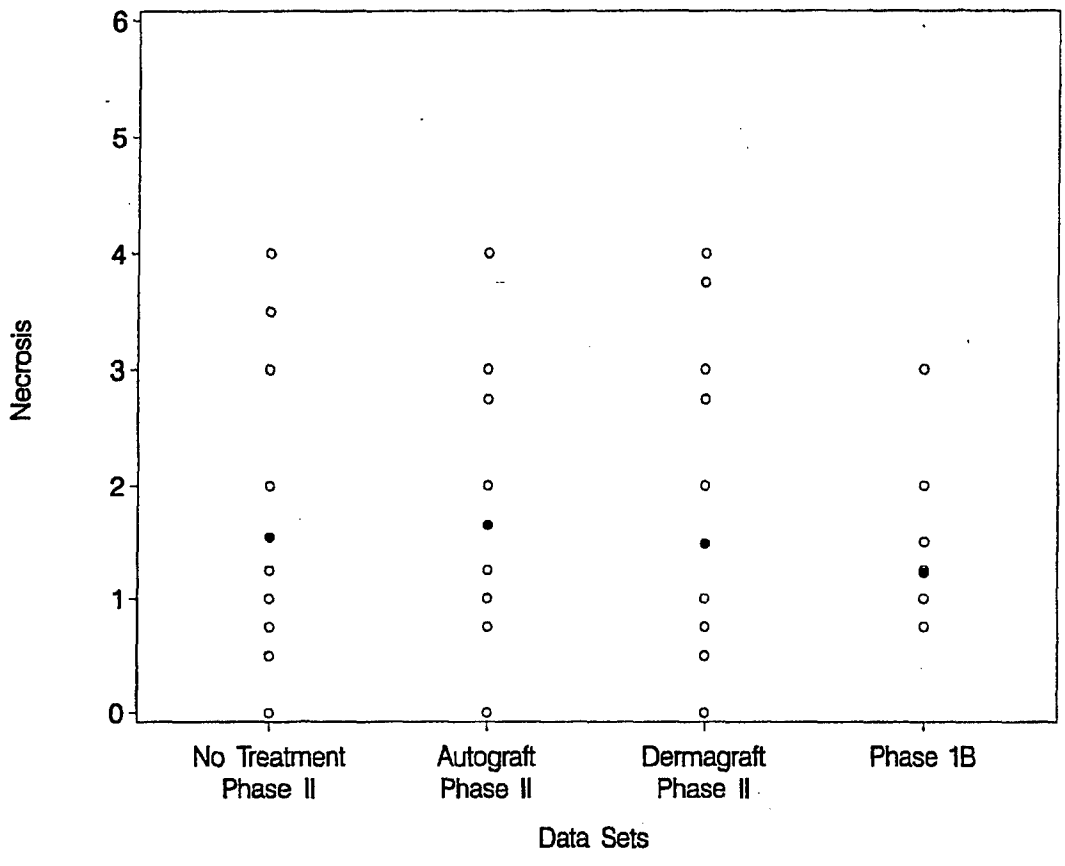

Figure 4. **Clinical Observation Necrosis on Study Day 2 in Phase II and Phase I, Part B. Mean Necrosis (.) Overlaid on Observed Values**

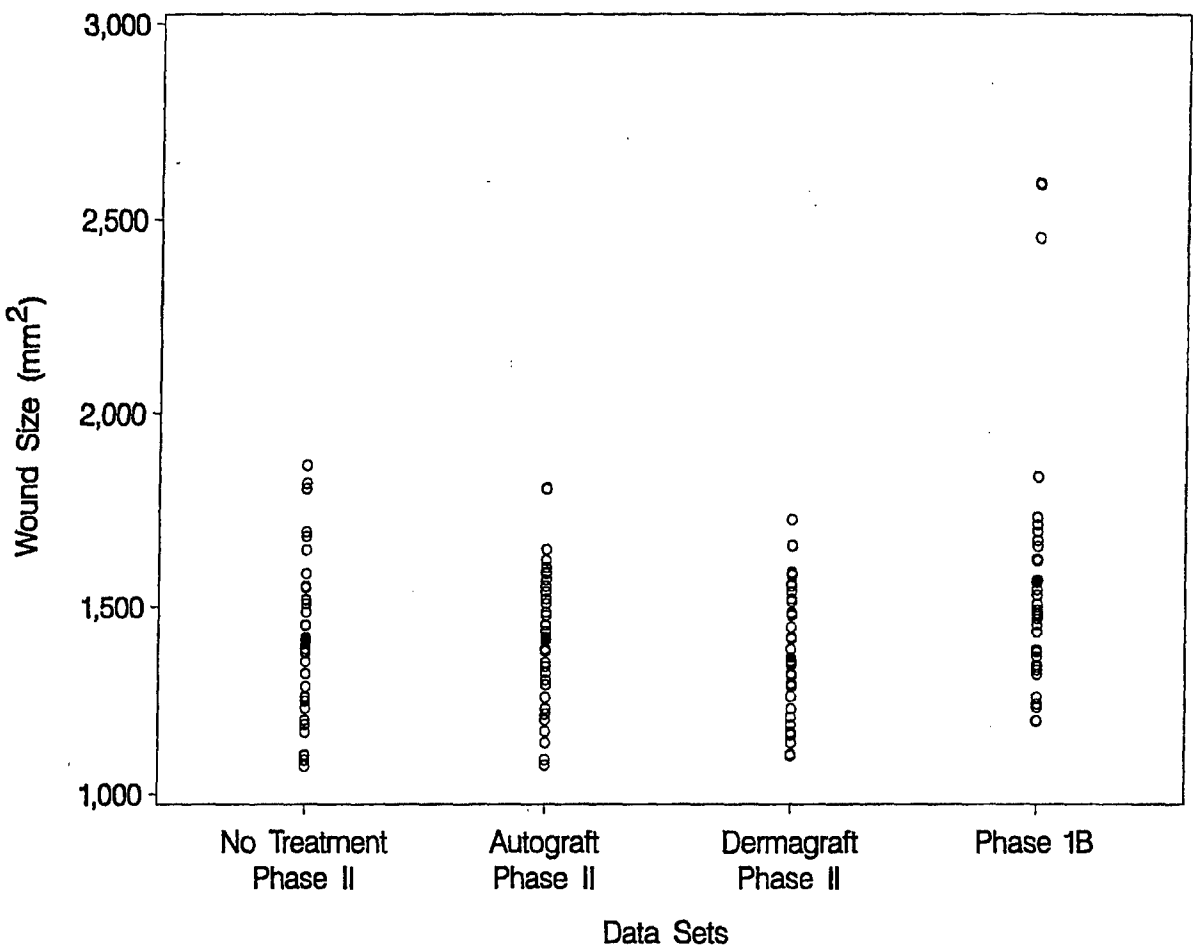

Figure 5. **Clinical Observation Wound Size on Study Day 2 in Phase II and Phase I, Part B. Mean Wound Size (.) Overlaid on Observed Values**

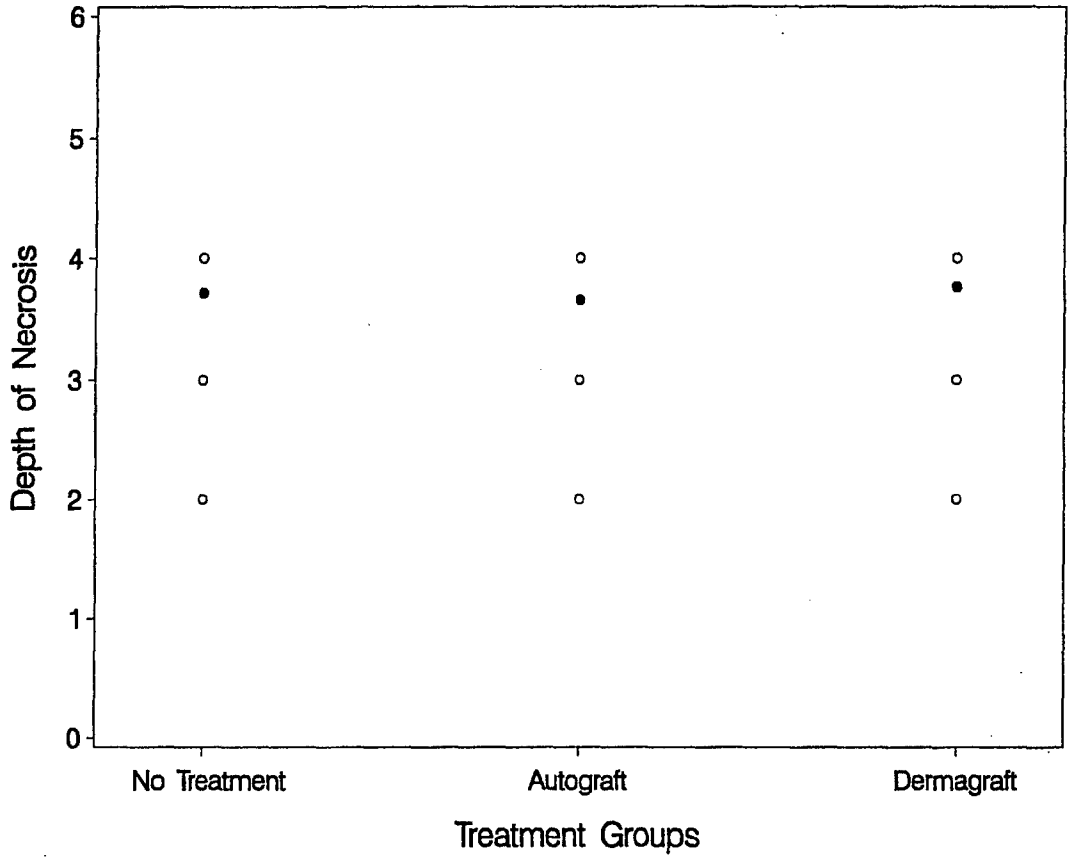

Figure 6. **Depth of Necrosis on Study Day 38. Mean Depth of Necrosis (•) Overlaid on Observed Values**

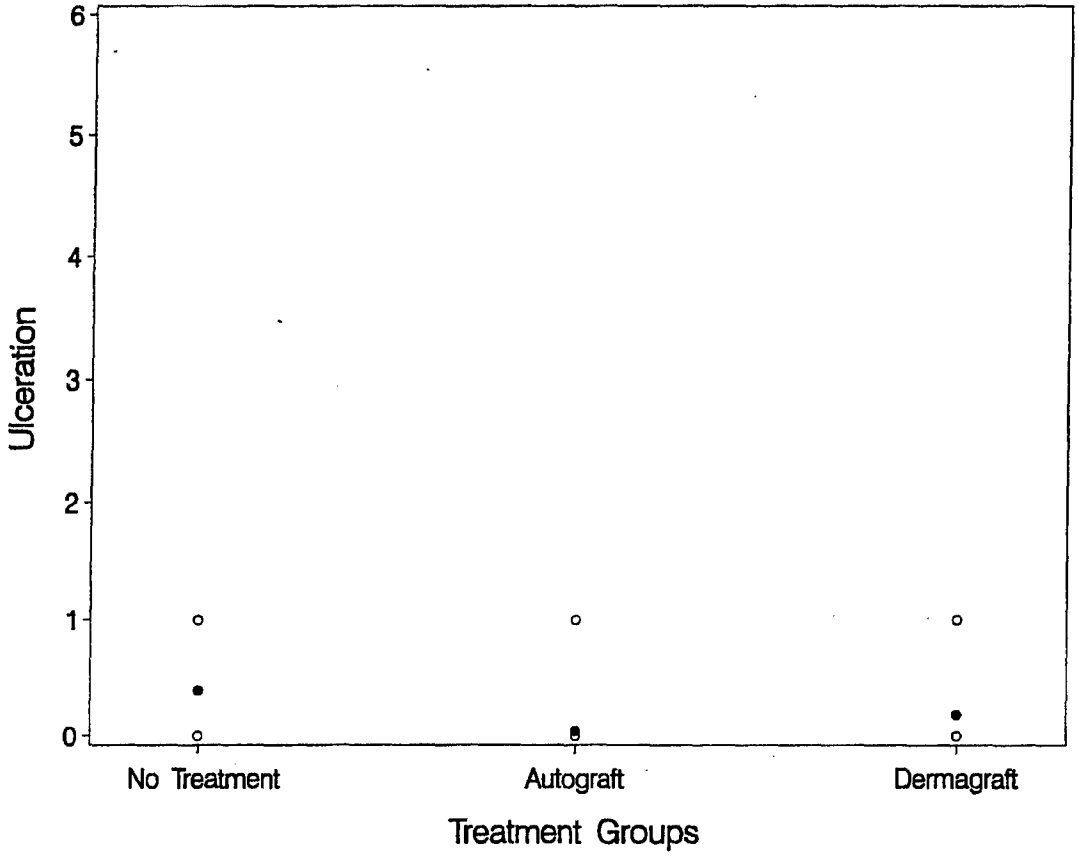

Figure 7. **Ulceration on Study Day 38. Mean Ulceration (•) Overlaid on Observed Values**

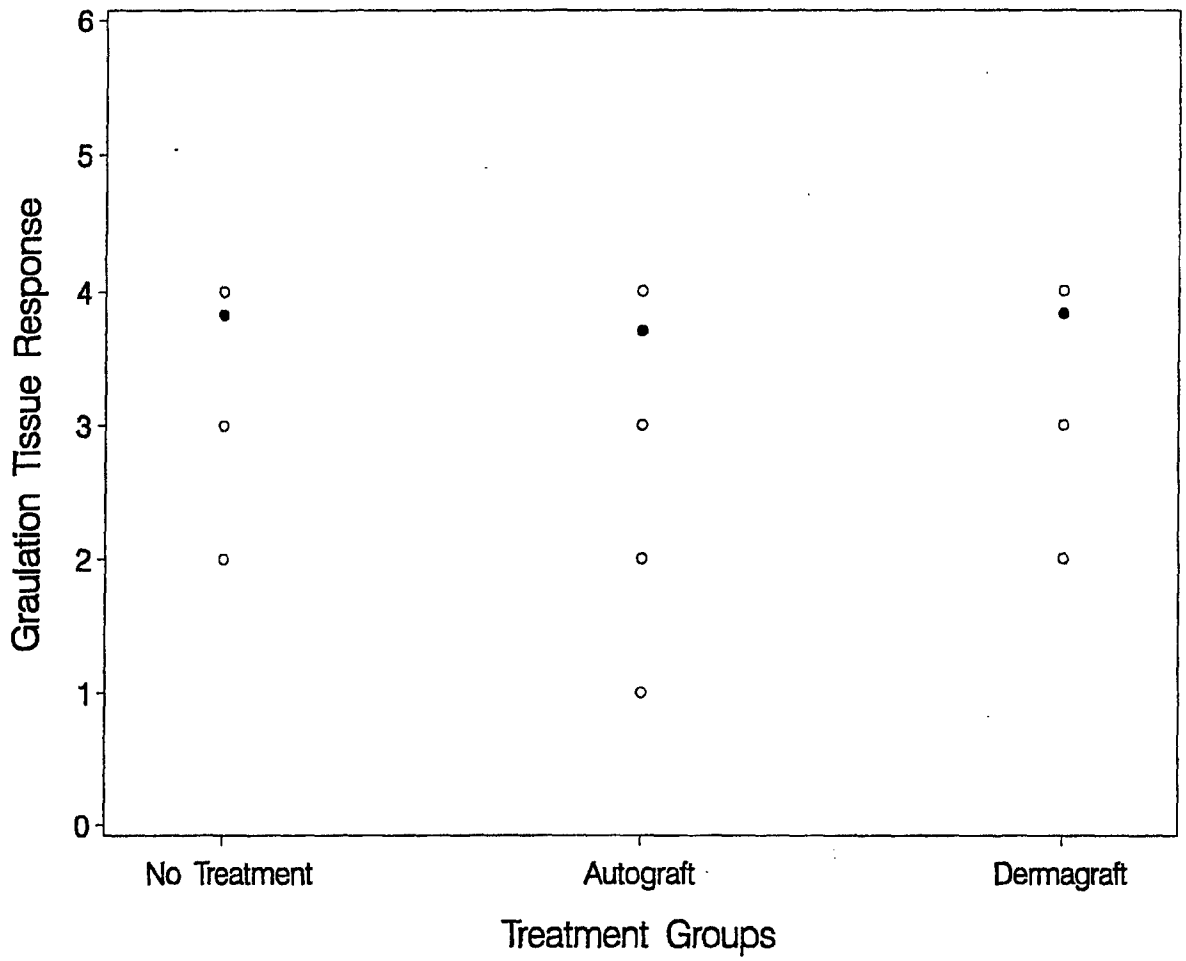

Figure 8. **Granulation Tissue Response on Study Day 38. Mean Granulation Tissue Response (•) Overlaid on Observed Values**

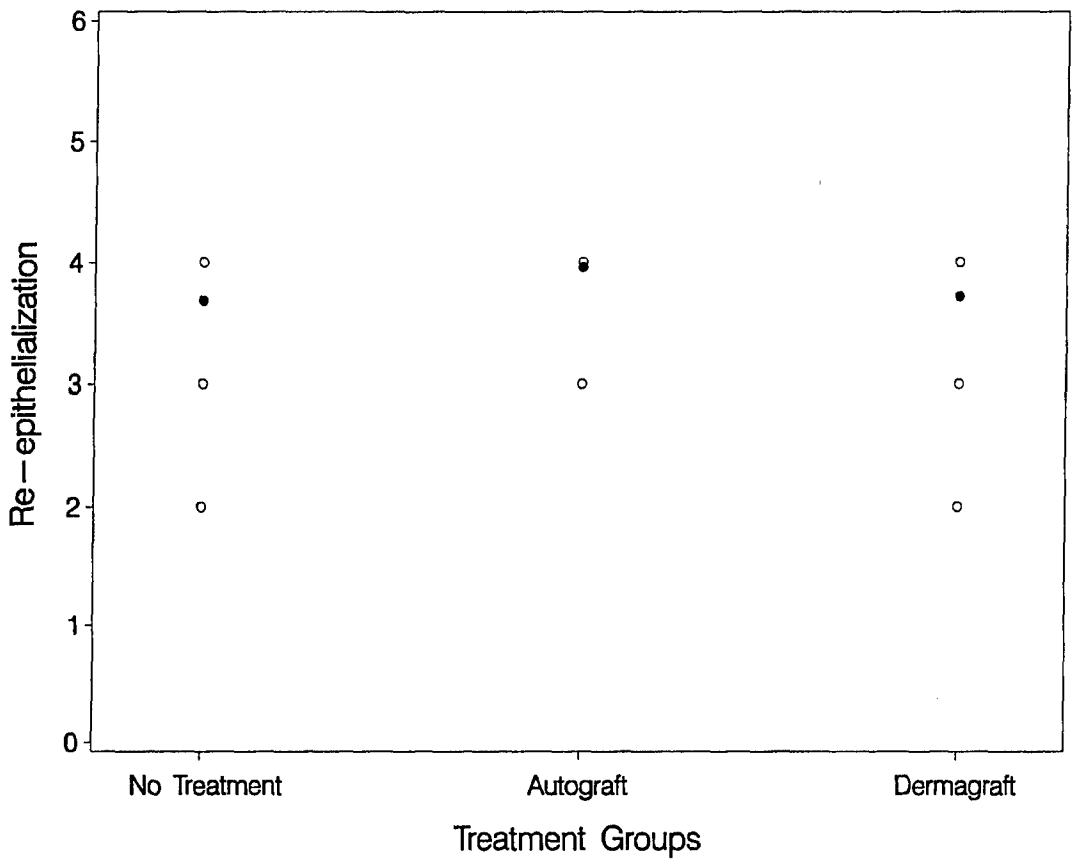

Figure 9. **Re-epithelialization on Study Day 38. Mean Re-epithelialization (•) Overlaid on Observed Values**

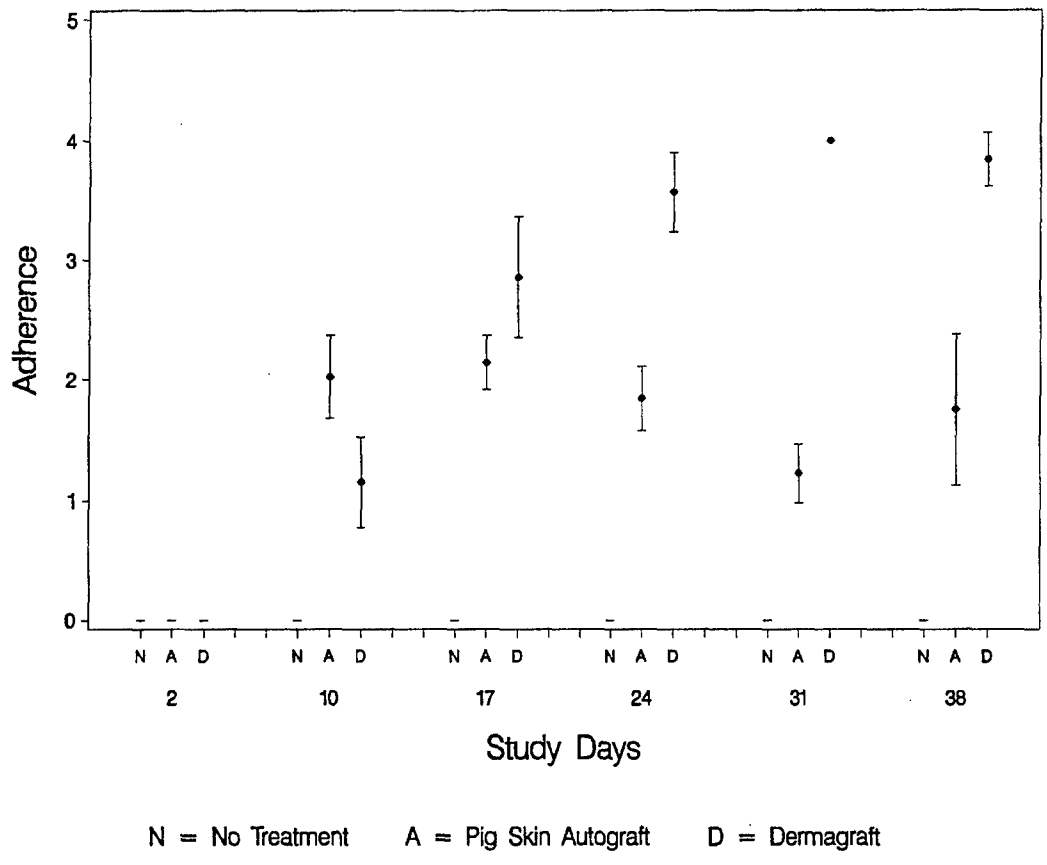

Figure 10. Mean Adherence and 95% Confidence Interval for Each Treatment Group and Study Day

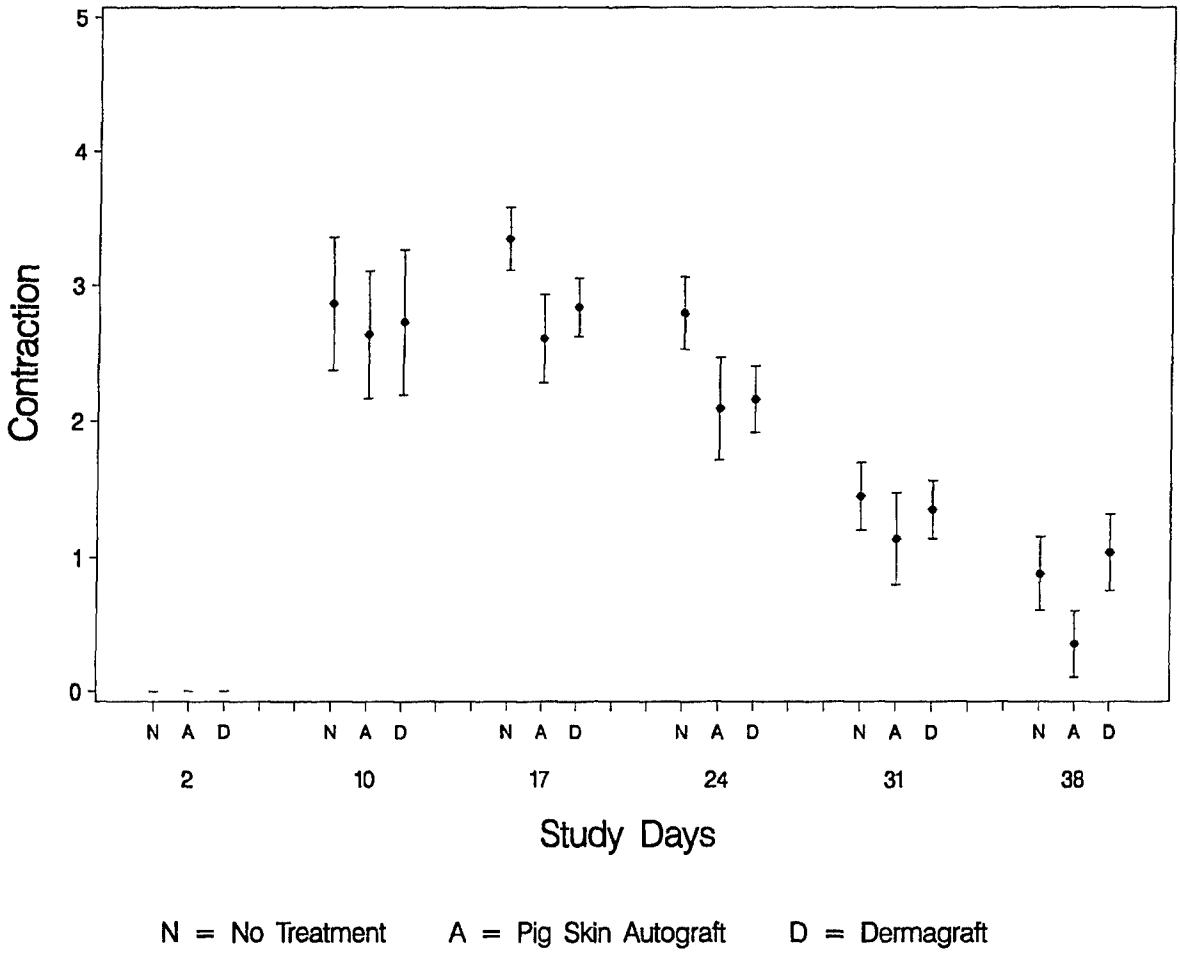

Figure 11. Mean Contraction and 95% Confidence Interval for Each Treatment Group and Study Day

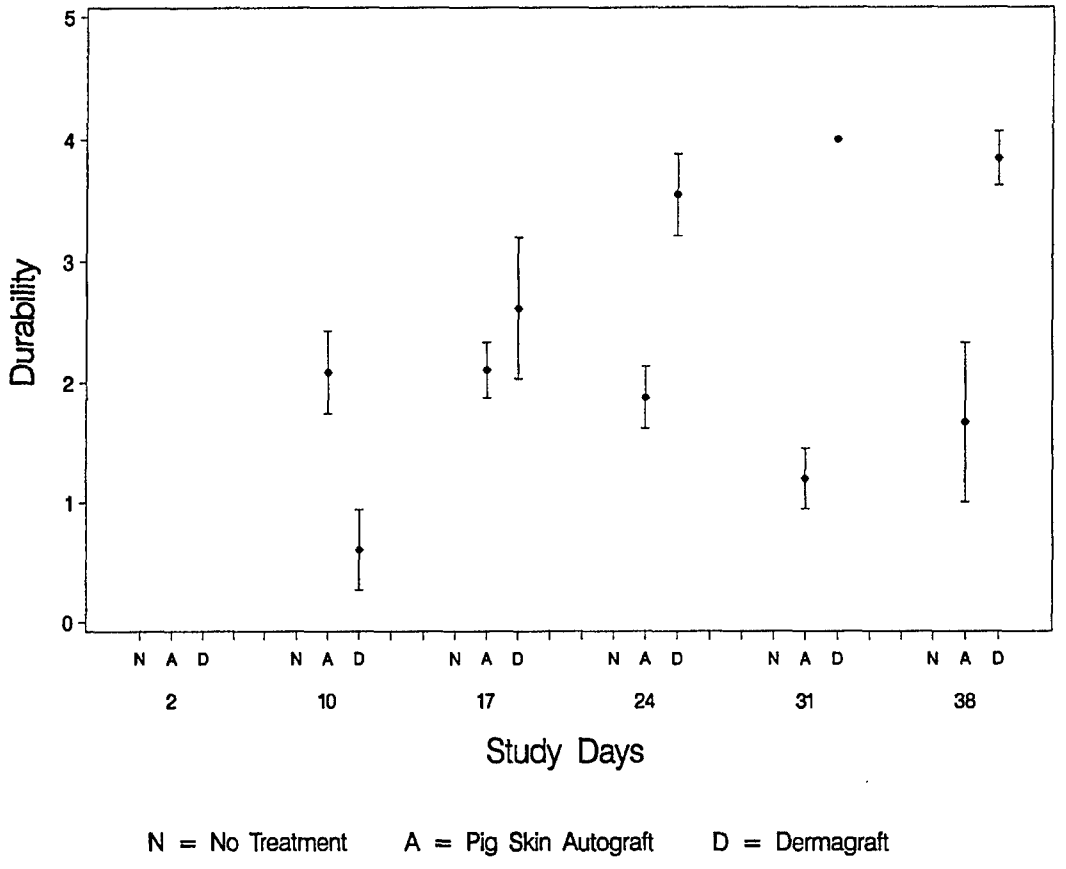

Figure 12. Mean Durability and 95% Confidence Interval for Each Treatment Group and Study Day

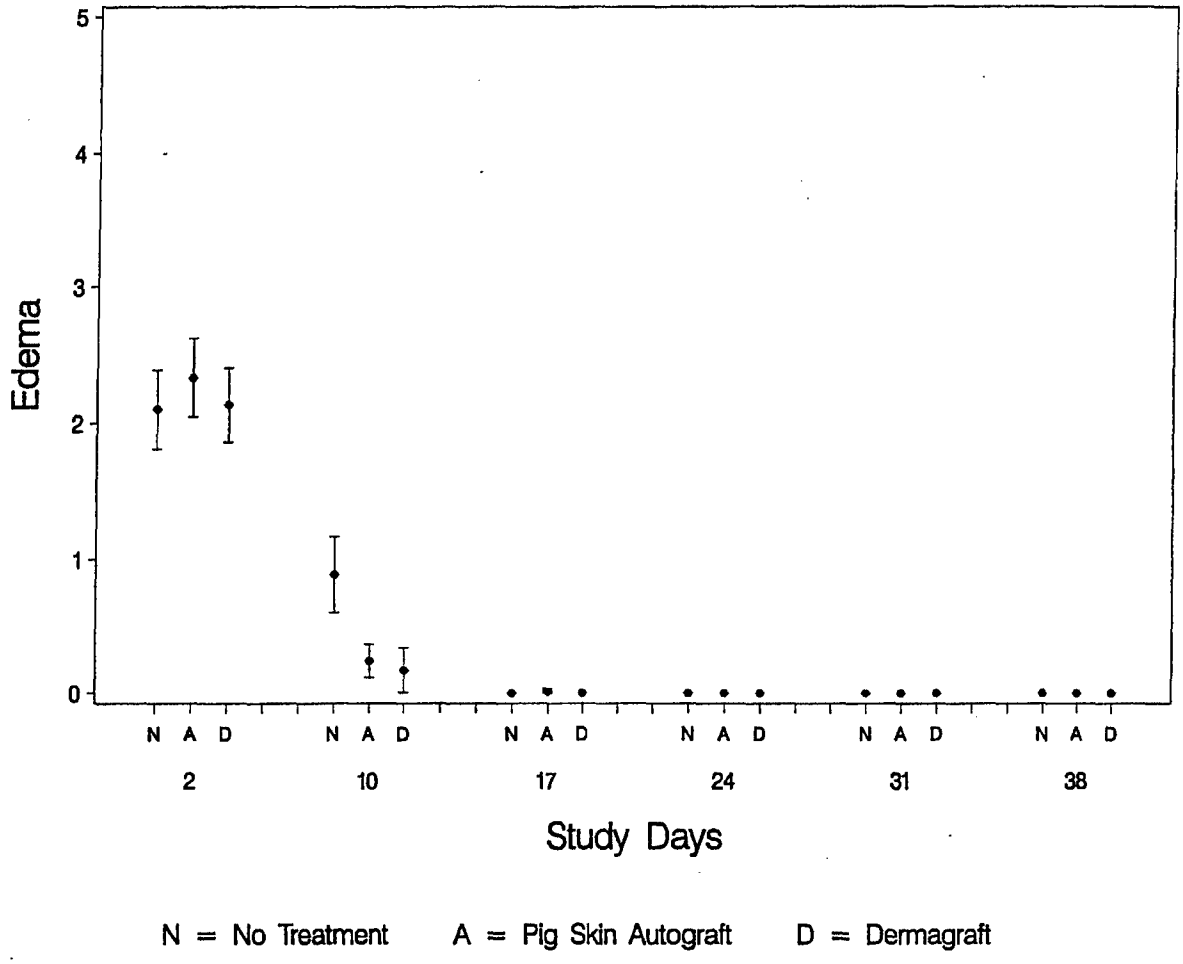

Figure 13. Mean Edema and 95% Confidence Interval for Each Treatment Group and Study Day

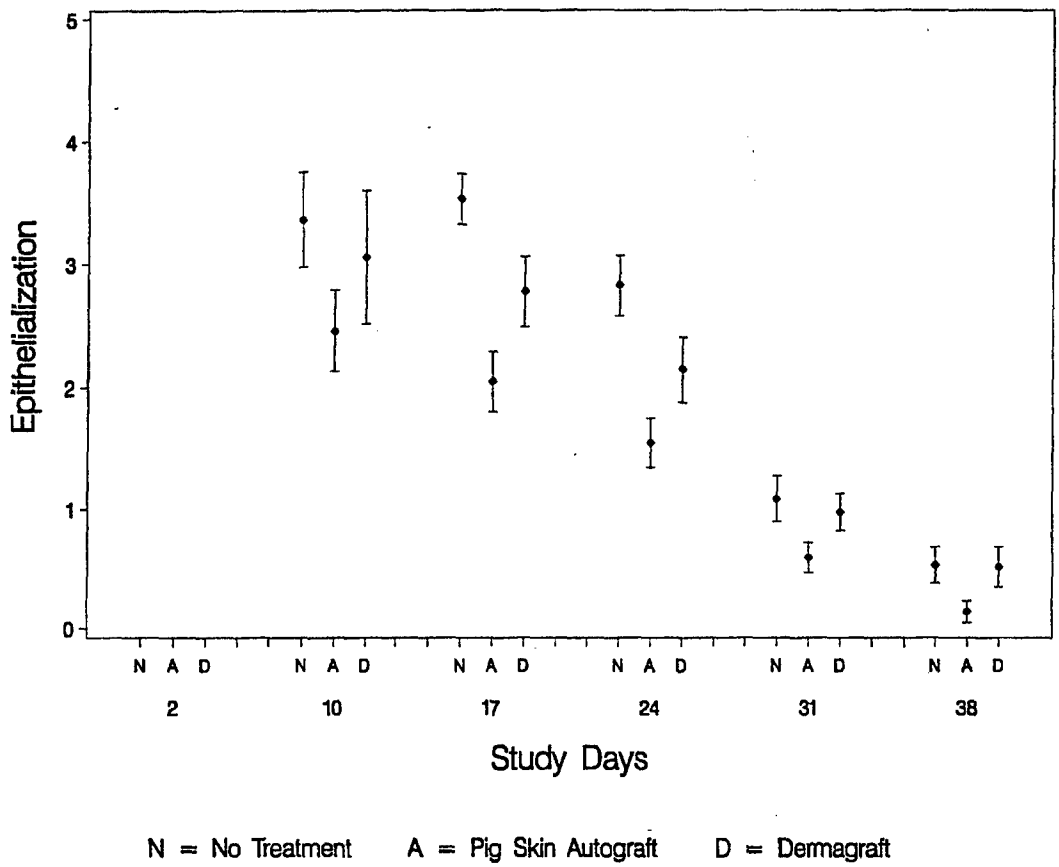

Figure 14. Mean Epithelialization and 95% Confidence Interval for Each Treatment Group and Study Day

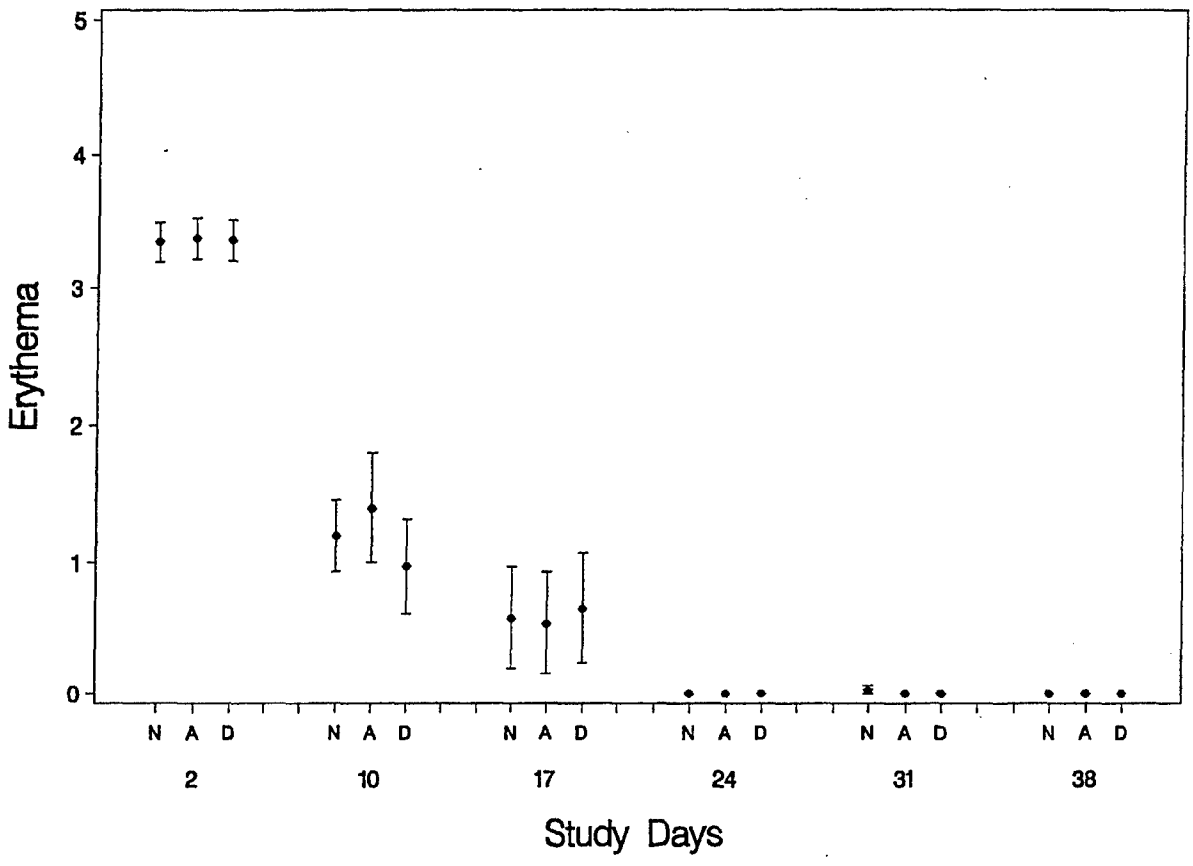

Figure 15. Mean Erythema and 95% Confidence Interval for Each Treatment Group and Study Day

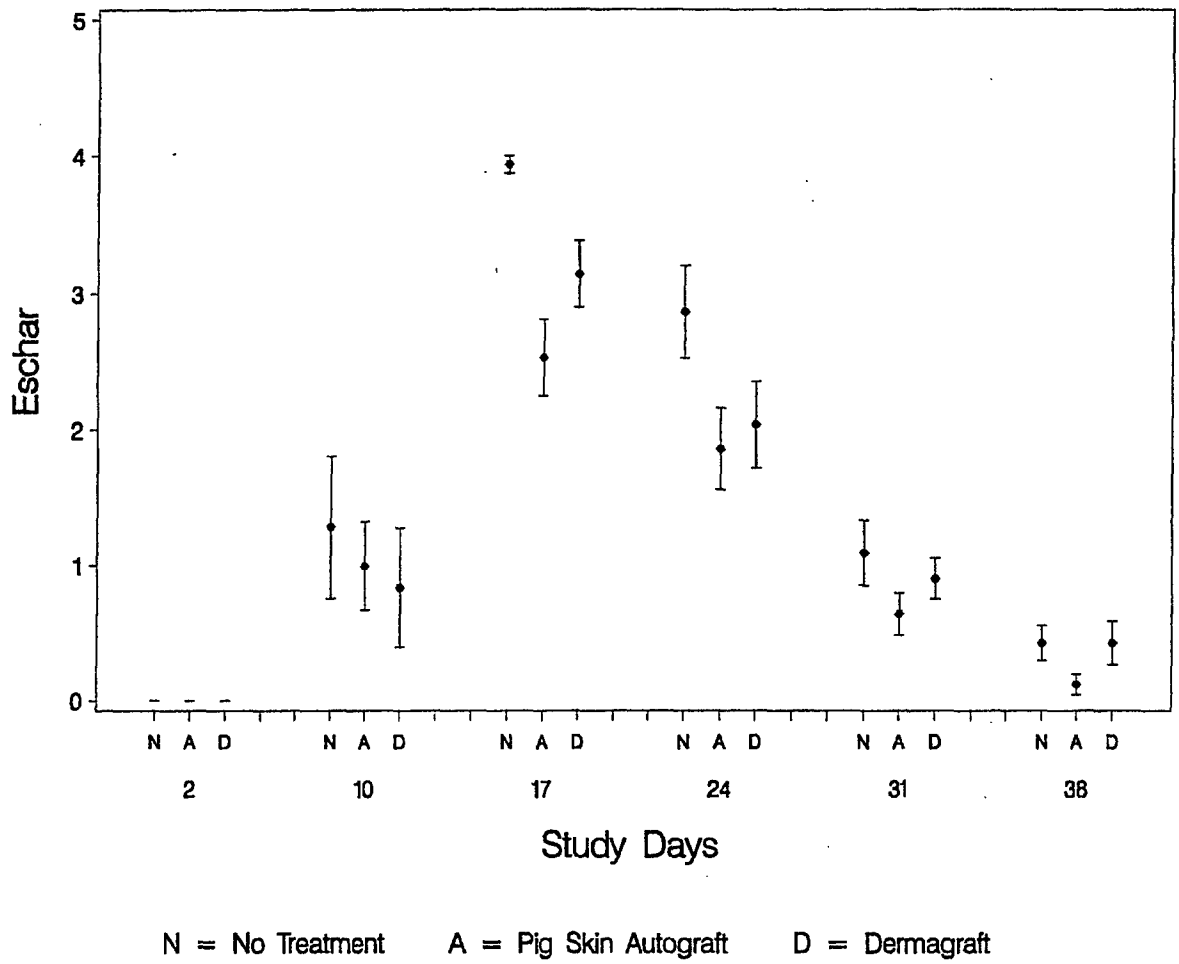

Figure 16. Mean Eschar and 95% Confidence Interval for Each Treatment Group and Study Day

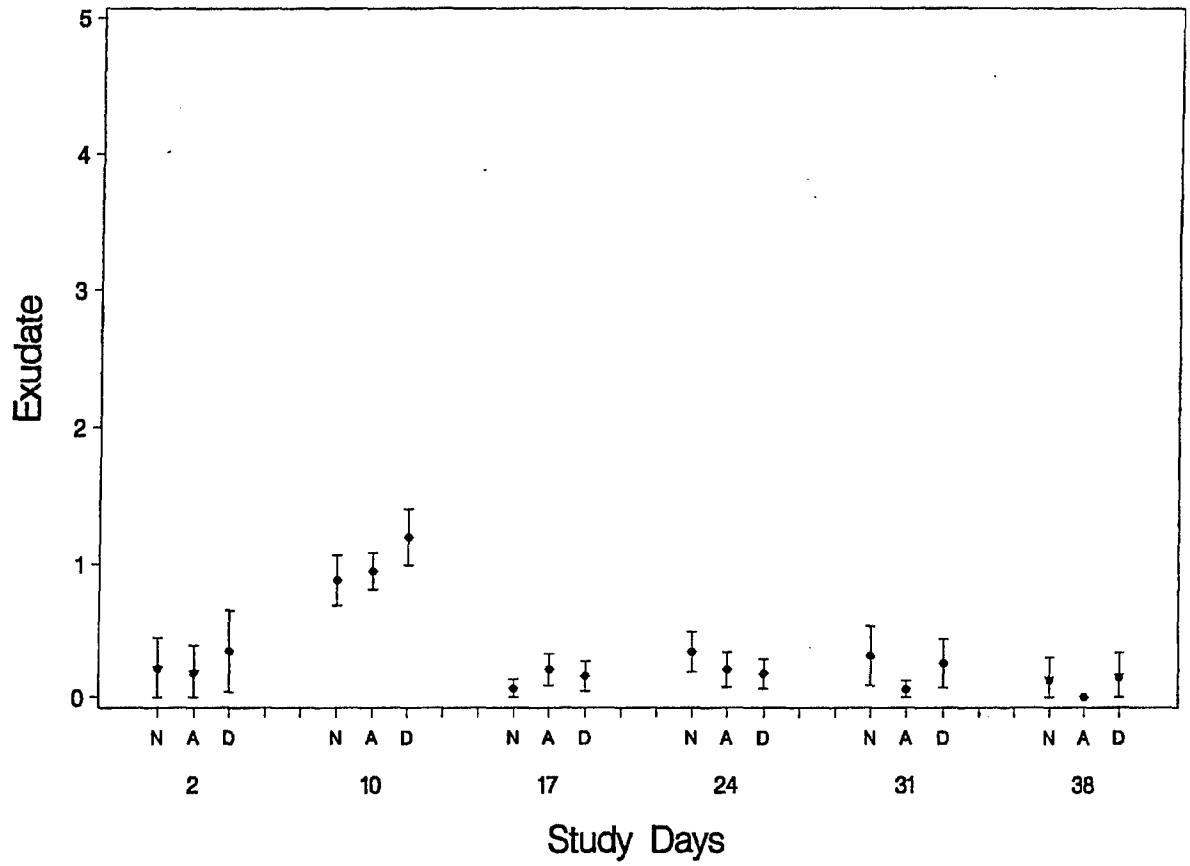

Figure 17. Mean Exudate and 95% Confidence Interval for Each Treatment Group and Study Day

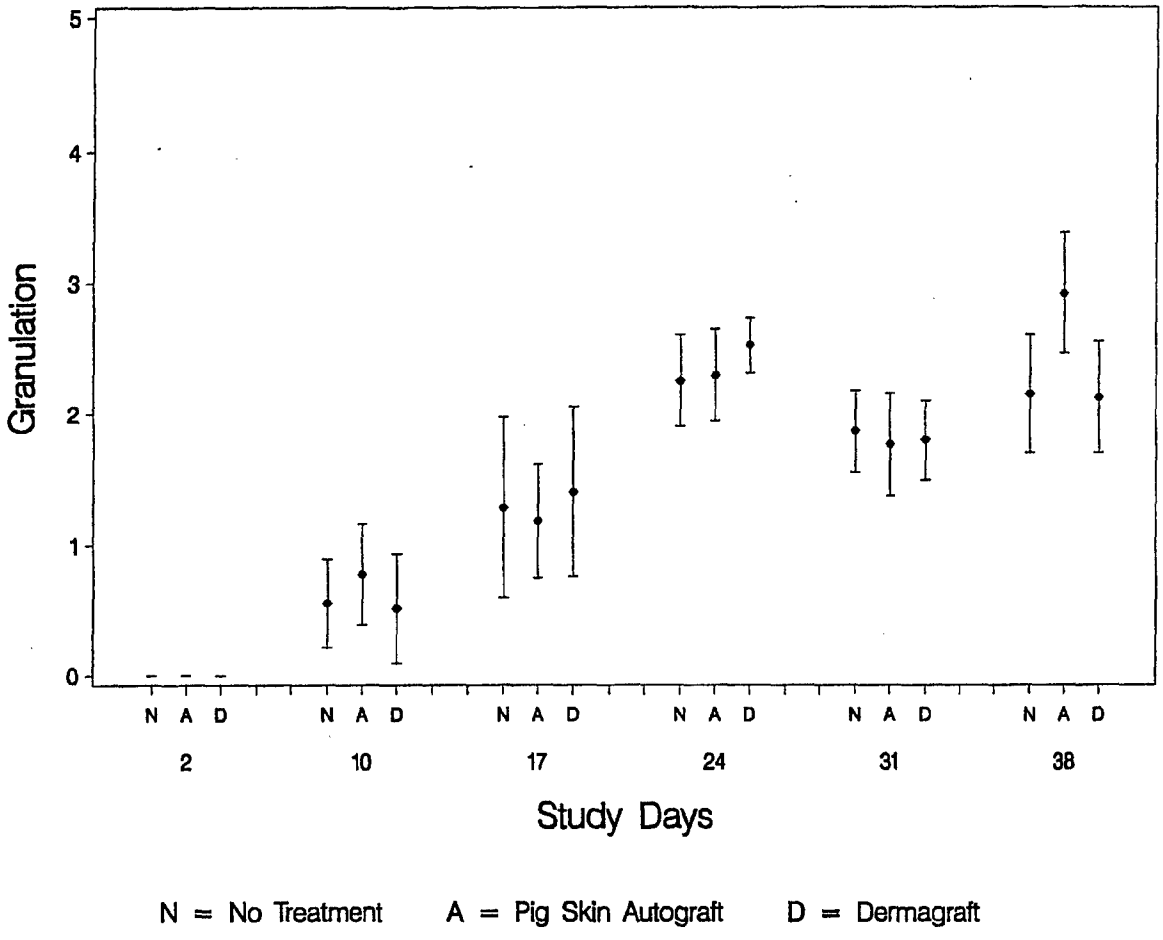

Figure 18. Mean Granulation and 95% Confidence Interval for Each Treatment Group and Study Day

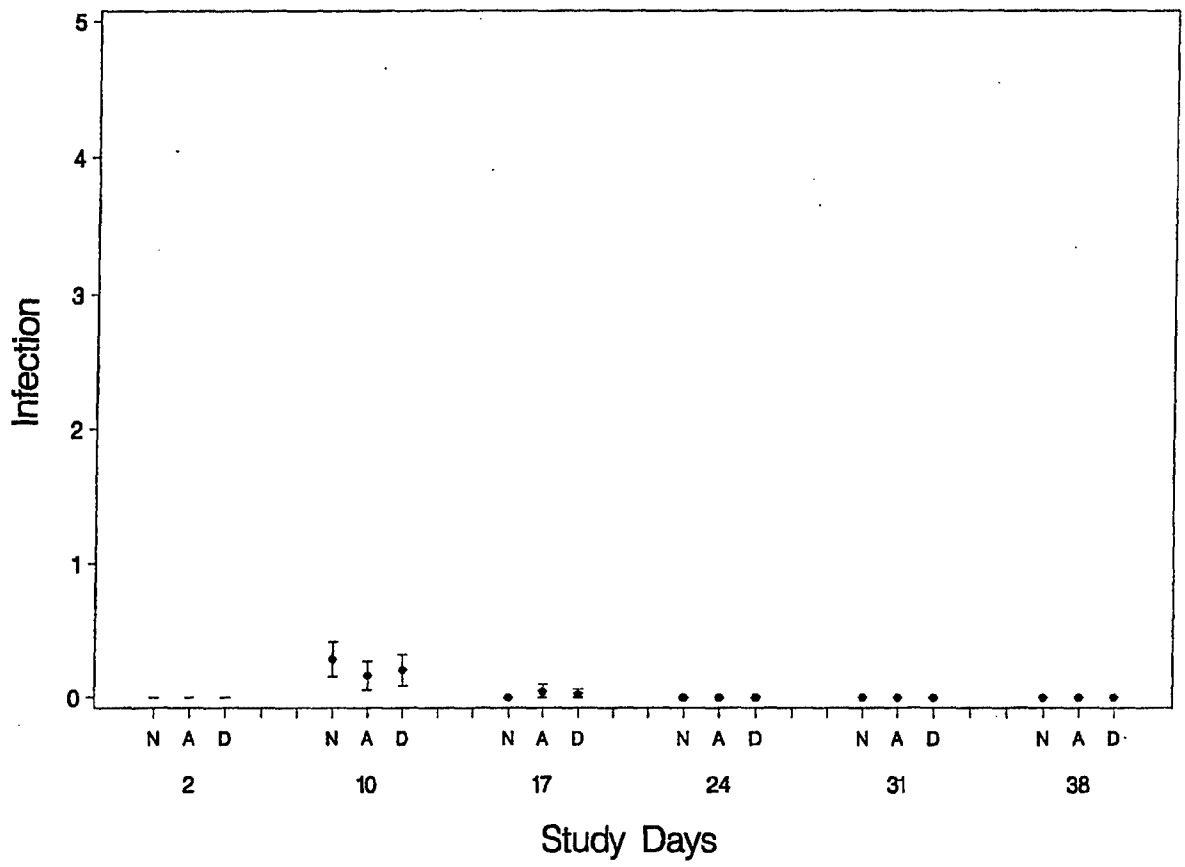

Figure 19. Mean Infection and 95% Confidence Interval for Each Treatment Group and Study Day

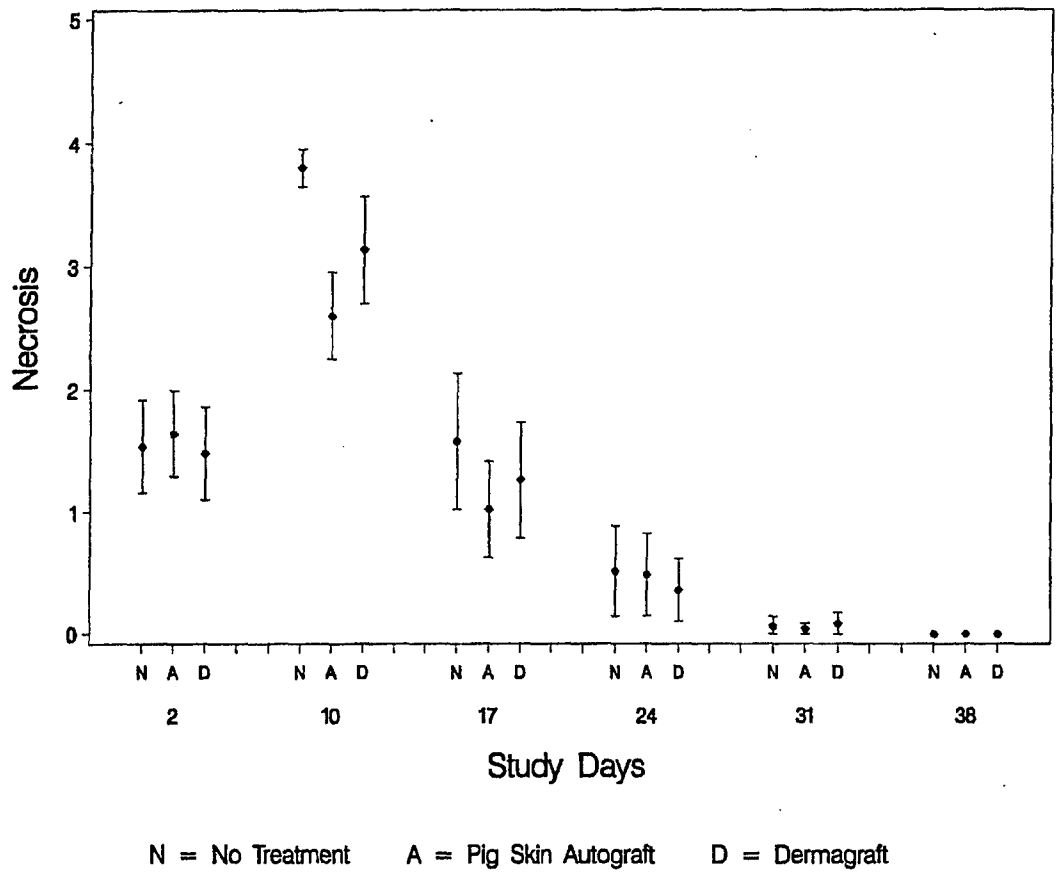

Figure 20. Mean Necrosis and 95% Confidence Interval for Each Treatment Group and Study Day

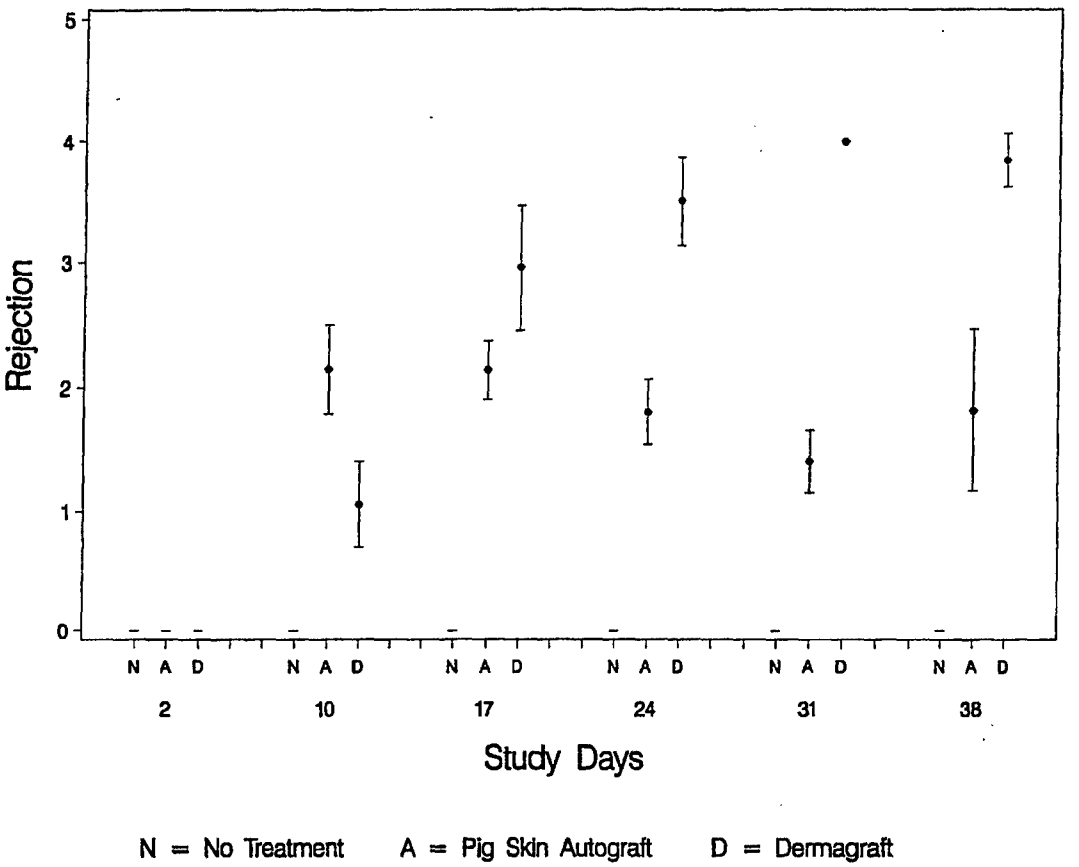

Figure 21. Mean Rejection and 95% Confidence Interval for Each Treatment Group and Study Day

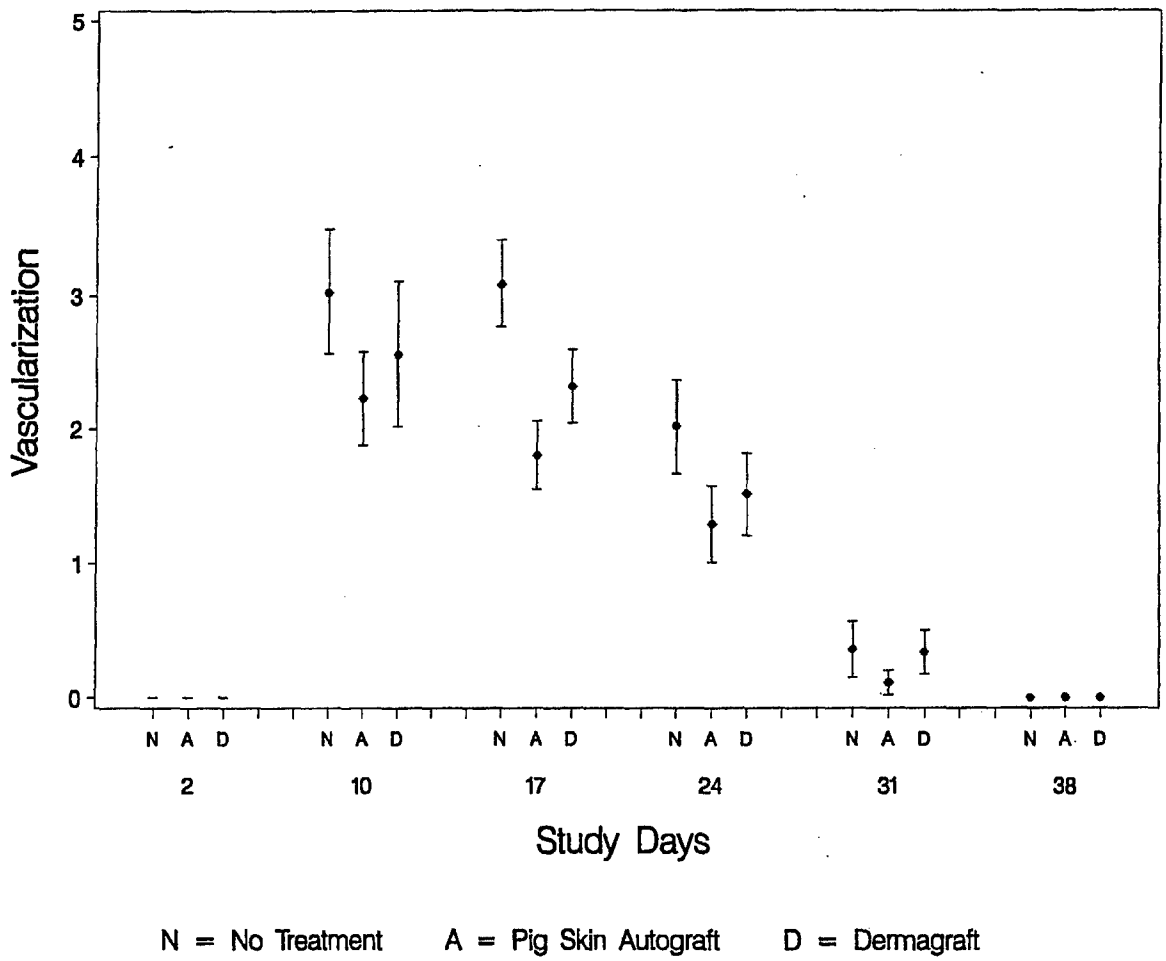

Figure 22. Mean Vascularization and 95% Confidence Interval for Each Treatment Group and Study Day

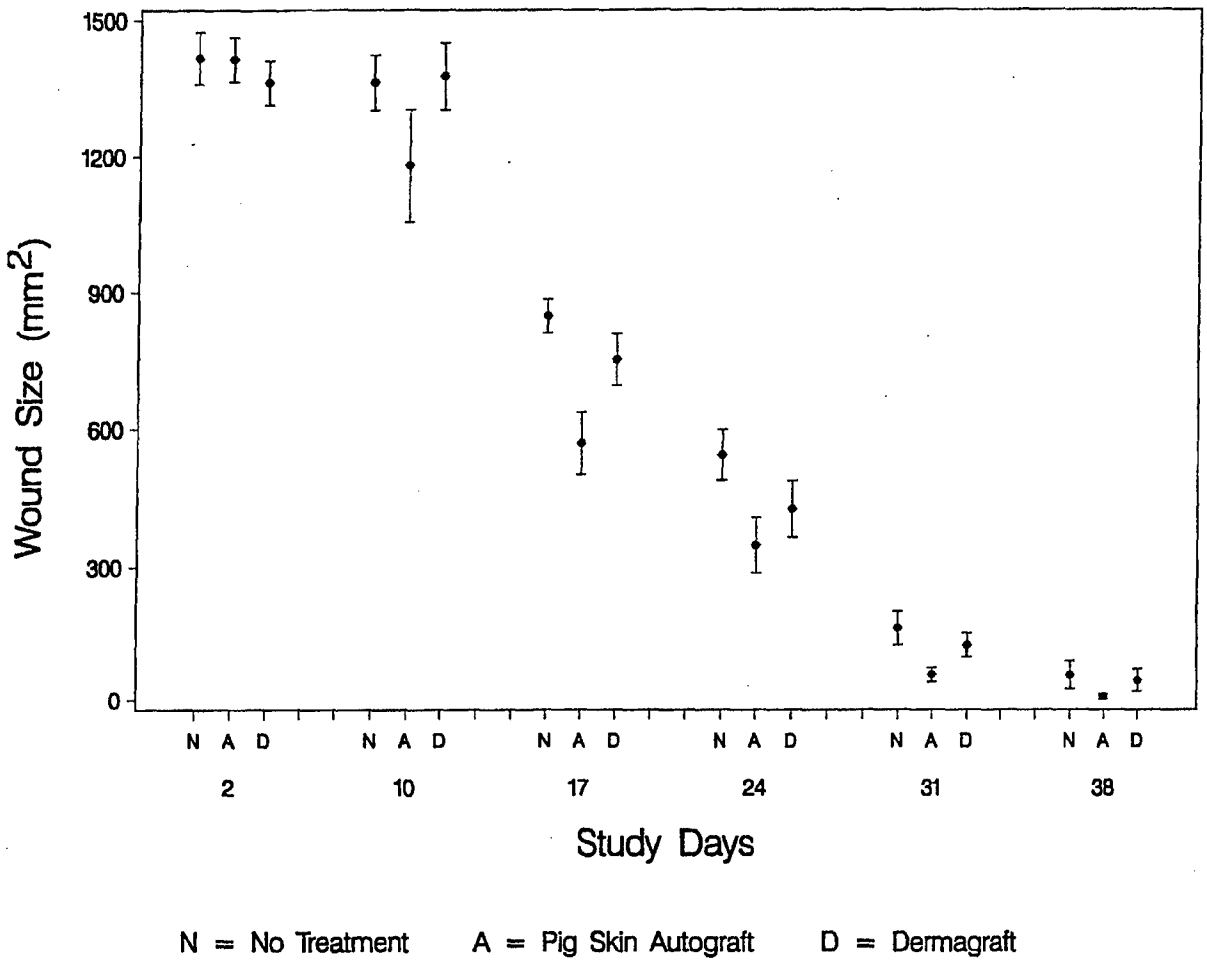

Figure 23. Mean Wound Size and 95% Confidence Interval for Each Treatment Group and Study Day

ATTACHMENT F

Phase III Statistics Report for Parts B and C

Battelle
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Project Number G1555-B33ASTAT (3104)

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s:\shum\Task 33\Phase IIIB\anesthesia report_new-Revised 6-15.doc

Date June 15, 2000

To **Frances Reid**

From Shawn Shumaker SMS

Subject **Statistical Analysis of Anesthesia Effects in MREF Task 94-33, Phase III, Part B**

Attached is a report describing the statistical analysis used to choose the anesthetic regimen with minimal effects or consistent vascular effects on dermal dose sites using Laser Doppler, Minolta Chromameter, and Evaporimeter instruments.

If you have any questions on this analysis, please contact me at 424-3232.

SMS:llj
Attachment

For Review and Approval

	Name	Initials	Date
Originator	Shawn Shumaker	SMS	6/15/2000
Concurrence	Jennifer Holdcraft	JRH	6/16/2000
	Nancy Niemuth	N	6/16/00
Approved	Ben Pierce	BKP	6/22/2000

Sent: Interoffice mail

MREF TASK 94-33, PHASE III, PART B

STATISTICAL REPORT ON ANESTHESIA EFFECTS

June 15, 2000

Introduction

Experiments were conducted under Phase III, Part B of MREF Task 94-33 to select an anesthetic regimen with minimal effects or consistent vascular effects on dermal dose sites as measured by Laser Doppler, Minolta Chromameter, and Evaporimeter instruments.

Three anesthesia regimens were to be tested in six animals using a cross-over design with a one-week washout between treatments. The following regimens were to be tested:

X: Repeated injections of Telazol/Xylazine combination to induce and maintain anesthesia;

Y: Initial injection of Telazol/Xylazine combination to induce anesthesia, followed by Isoflurane inhalation to maintain anesthesia; and

Z: Isoflurane inhalation to induce and maintain anesthesia, with animals masked down.

Anesthesia regimen Z was dropped after the first 2 animals were tested, due to practical considerations, and anesthesia regimen W was added as a replacement:

W: Anesthesia induced by Xylazine injection and maintained by repeated Ketamine injections.

Although unavoidable, this action compromised the crossover design, as anesthesia regimen W was tested last for each animal.

Six female Yorkshire pigs (6 ventral sites per pig) were used for the anesthesia experiments. Each anesthesia regimen was administered in three iterations within a week (two days apart), with a minimum one-week washout period before the next anesthesia regimen was tested. On each day of testing, each of six sites per animal was evaluated in four successive testing rounds using Laser Doppler, Minolta Chromameter, and Evaporimeter instruments. A single reading was taken during each round for Laser Doppler and Evaporimeter. For the Minolta Chromameter, four replicate readings were taken and averaged to give one reading for each round.

Table 1 illustrates the course of the anesthesia experiments for the six animals tested. As shown in Table 1, animals 99-23-12 and 99-55-6 received the minimum one-week washout between anesthesia regimens. Animals 99-2-10 and 99-2-9 received anesthesia Y followed by X, and then were idle for 2 weeks before receiving anesthesia W. Animals 99-2-11 and 99-6-1 received

animals were on study (relative to their first anesthesia application) for 2-3 weeks longer than the other animals.

Table 1. Time Course of Anesthesia Experiments for Each Animal

Date	Animal Number					
	1 99-23-12	2 99-55-6	3 99-2-10	4 99-2-11	5 99-2-9	6 99-6-1
February 10	X	X				
February 12	X	X				
February 14	X	X				
February 15			Y		Y	
February 16				Z		Z
February 17			Y		Y	
February 18				Z		Z
February 19			Y		Y	
February 20				Z		Z
February 22	Y	Y				
February 24	Y	Y				
February 26	Y	Y				
March 1			X		X	
March 2				Y		Y
March 3			X		X	
March 4				Y		Y
March 5			X		X	
March 6				Y		Y
March 8	W	W				
March 10	W	W				
March 12	W	W				
March 22			W		W	
March 23				X		X
March 24			W		W	
March 25				X		X
March 26			W		W	
March 27				X		X
March 30			• X		• X	
April 6				W		W
April 8				W		W
April 10				W		W
April 14				• X		• X

• Only Evaporimeter readings were taken, Not used in primary analysis

Methods

Histograms of each of the response variables revealed that the distribution of each was bell-shaped and adequately approximated by a normal distribution. Once this determination was made, mixed models were then fit to each of the responses.

To determine the anesthetic regimen with the least effect on a response variable, mixed analysis-of-variance (ANOVA) models (with both fixed and random effects) were fitted separately to the Laser Doppler, Chromameter, and Evaporimeter data, using the MIXED procedure in SAS® (V8). For Chromameter data, data from the four replicate readings were averaged. Only main effects and interactions that were statistically significant at the 0.05 level were included in the models. Several main effects were of interest: anesthetic regimen (X, Y, or

Z), sampling iteration (1 through 3), ventral site on animal (six sites), and round within sampling iteration (four rounds of sampling conducted on each sampling iteration). In addition, an interaction between anesthesia and sampling iteration was present for both Chromameter and Laser Doppler responses. The random effect was animal, which assumes independence between animals. An interaction between sampling iteration and animal was present for all responses and was included as an additional random effect. A list of effects in the mixed models for each instrument is given in Table 2. To determine the anesthesia with the least effect, statistical contrasts of the parameter estimates were used to determine if there were significant differences in mean response levels for the three anesthesia regimens, while controlling for other factors in the model. Model-based profile plots were produced to aid in the interpretation of the model.

Table 2. Effects in Fitted Model for Laser Doppler, Evaporimeter, and Chromameter Data

Response	General Fixed Effects and their Interactions in Mixed Model	Random Effects in Mixed Model	Additional Effects in Mixed Model
Laser Doppler	• Anesthesia • Sampling Iteration • Ventral Site • Sampling Iteration × Sampling Round	• Animal • Animal × Sampling Iteration	• Anesthesia × Sampling Iteration
Evaporimeter			• None
Chromameter			• Anesthesia × Sampling Iteration

Results

The models were used to estimate responses for the Laser Doppler, Evaporimeter, and Chromameter responses by anesthesia, iteration, and round. These results can be seen in Table 3 and Figures 1 through 3. Table 4 and Figure 4 show the estimated response when anesthesia X, Y, or W are administered. These tables and figures demonstrate that

- For the Laser Doppler readings, anesthesia W yielded the highest response and anesthesia Y yielded the lowest response. As indicated in Table 4 and Figure 4, the mean responses were significantly different for all three anesthesia regimens. The first sampling round had the highest response for each sampling iteration. Mean response declined on later study days for regimens X and W, but not for Y (Figure 1).

- For Evaporimeter, anesthesia W yielded the lowest response on average and anesthesia X and Y yielded higher responses (Table 4 and Figure 4). The first sampling round had the highest response and the fourth sampling round had the lowest response. Mean responses declined over three days of experiments for each anesthesia (Figure 2).

- For Chromameter, anesthesia W yielded the lowest response and anesthesia X and Y yielded higher responses, which were not statistically significantly different from each other on average (Table 4 and Figure 4). The first sampling round had the highest response for all three regimens, while later rounds had lower responses. Regimens X and W had consistent readings over 3 days while regimen Y had greater day-to-day variability (Figure 3).

Conclusions

For Laser Doppler, the three anesthesias yielded a significantly different response level, but none was preferred. The anesthesias behaved similarly within a day. Anesthesia X and W declined over multiple treatment days, while Y was less consistent. No regimen was clearly preferred for the Laser Doppler instrument.

For the Evaporimeter instrument, Anesthesia W yielded a lower response than X and Y. There was a decline in response over time within day that indicated that Evaporimeter was sensitive to the time post-anesthesia. The response also declined with multiple treatment days for each anesthesia. Regimen X or Y was preferred for Evaporimeter readings.

For the Chromameter instrument, anesthesias X and Y yielded higher response than W indicating a greater blanching effect for anesthesia W. The anesthesias behaved similarly within a day with round one yielding a higher response than subsequent rounds indicating that Chromameter was sensitive to the time post-anesthesia. Response over multiple days was less variable for anesthesia X and W than for Y. Either regimen X or W was suitable, with X preferred due to lesser blanching effect.

Regimen X was recommended for future experiments as it is the most suitable when readings are to be taken from all 3 instruments. It was also recommended that Chromameter and Evaporimeter readings be taken first as they are most sensitive to time post-anesthesia.

Table 3. Model Estimated Response by Anesthesia, Iteration, and Round for Laser Doppler, Evaporimeter, and Chromameter Instruments

Response	Anesthesia	Iteration	Estimated Response (SE)			
			Round 1	Round 2	Round 3	Round 4
Laser Doppler	W	1	698.55 (32.72)	637.89 (32.72)	642.22 (32.72)	655.58 (32.76)
		2	618.27 (32.71)	618.49 (32.71)	602.21 (32.71)	607.47 (32.71)
		3	570.57 (32.76)	570.29 (32.72)	566.04 (32.72)	570.12 (32.72)
	X	1	661.46 (32.71)	600.81 (32.71)	605.13 (32.71)	618.49 (32.72)
		2	586.90 (32.71)	587.11 (32.71)	570.83 (32.71)	576.09 (32.71)
		3	564.95 (32.72)	564.67 (32.71)	560.43 (32.71)	564.51 (32.71)
	Y	1	540.73 (32.71)	480.07 (32.71)	484.40 (32.71)	497.76 (32.72)
		2	563.49 (32.71)	563.71 (32.71)	547.42 (32.71)	552.68 (32.71)
		3	512.09 (32.72)	511.81 (32.71)	507.56 (32.71)	511.64 (32.71)
Evaporimeter	W	1	11.46 (0.46)	10.59 (0.46)	10.59 (0.46)	10.01 (0.46)
		2	9.61 (0.46)	9.05 (0.46)	8.82 (0.46)	8.45 (0.46)
		3	8.58 (0.46)	7.82 (0.46)	7.73 (0.46)	7.71 (0.46)
	X	1	13.18 (0.46)	12.32 (0.46)	12.31 (0.46)	11.74 (0.46)
		2	11.33 (0.46)	10.77 (0.46)	10.54 (0.46)	10.17 (0.46)
		3	10.30 (0.46)	9.54 (0.46)	9.46 (0.46)	9.43 (0.46)
	Y	1	13.49 (0.46)	12.62 (0.46)	12.62 (0.46)	12.04 (0.46)
		2	11.63 (0.46)	11.08 (0.46)	10.85 (0.46)	10.48 (0.46)
		3	10.60 (0.46)	9.85 (0.46)	9.76 (0.46)	9.73 (0.46)
Chromameter	W	1	8.01 (0.37)	7.56 (0.37)	7.36 (0.37)	7.38 (0.37)
		2	8.54 (0.38)	8.32 (0.38)	8.16 (0.38)	8.37 (0.38)
		3	8.14 (0.38)	7.61 (0.38)	7.38 (0.38)	7.44 (0.38)
	X	1	9.77 (0.37)	9.32 (0.37)	9.11 (0.37)	9.14 (0.37)
		2	9.47 (0.37)	9.25 (0.37)	9.10 (0.37)	9.30 (0.37)
		3	9.34 (0.37)	8.80 (0.37)	8.58 (0.37)	8.64 (0.37)
	Y	1	9.44 (0.37)	8.99 (0.37)	8.79 (0.37)	8.81 (0.37)
		2	8.53 (0.37)	8.31 (0.37)	8.16 (0.37)	8.36 (0.37)
		3	10.24 (0.37)	9.70 (0.37)	9.48 (0.37)	9.54 (0.37)

F-6

Table 4. Estimated Response for each Anesthesia Regimen

Response	Anesthesia X: Estimated Response (SE)	Anesthesia Y: Estimated Response (SE)	Anesthesia W: Estimated Response (SE)	Difference in Response (P-Value)		
				X-Y	X-W	Y-W
Laser Doppler	588.45 (22.86)	522.78 (22.86)	613.14 (22.87)	65.67 (<.001)	-24.69 (0.007)	-90.36 (<.001)
Evaporimeter	10.92 (0.32)	11.23 (0.32)	9.20 (0.32)	-0.31 (0.038)	1.72 (<.001)	2.03 (<.001)
Chromameter	9.15 (0.30)	9.03 (0.30)	7.86 (0.30)	0.12 (0.214)	1.30 (<.001)	1.17 (<.001)

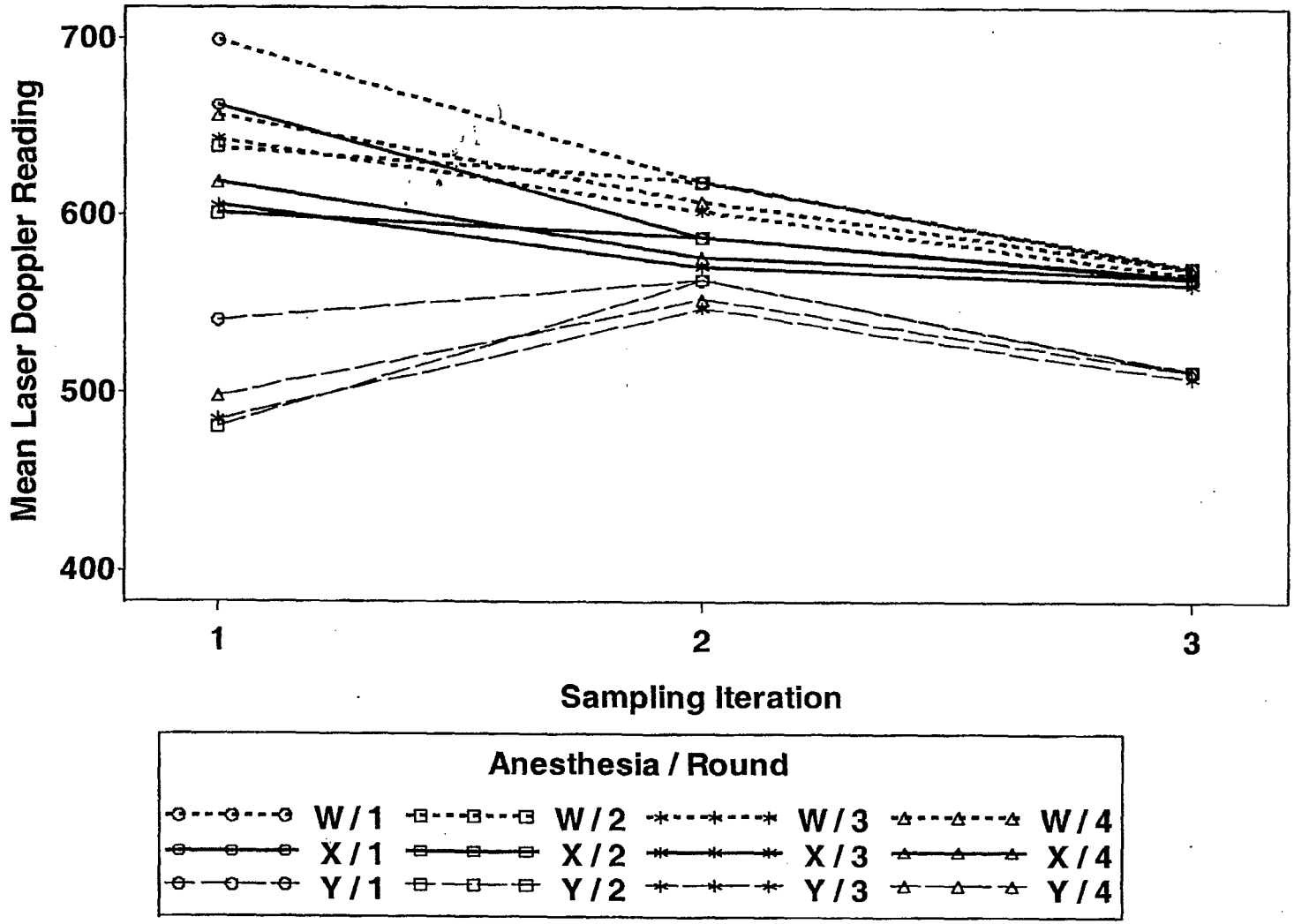

Figure 1. Estimated Response by Anesthesia, Iteration, and Round for Laser Doppler

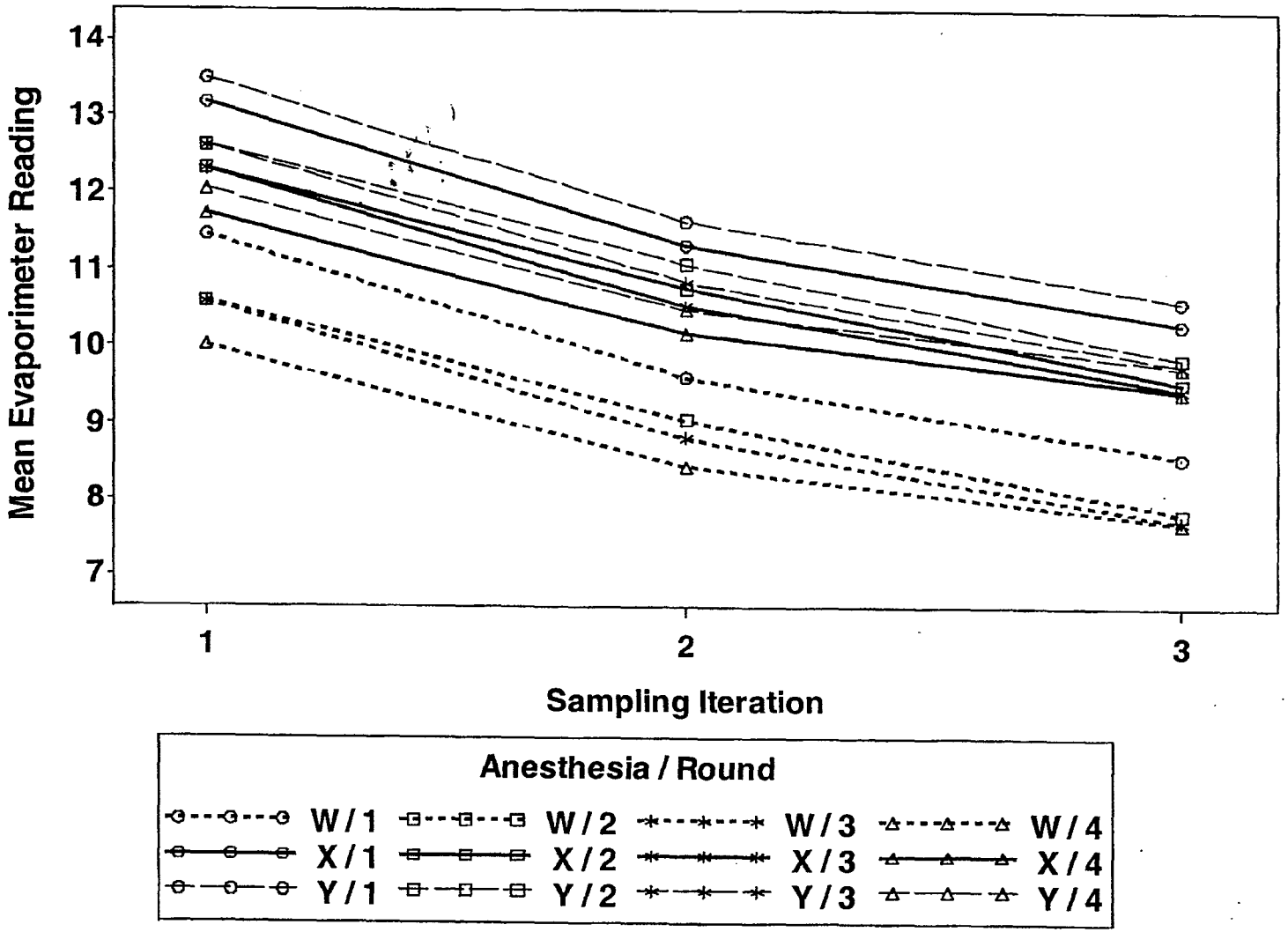

Figure 2. Estimated Response by Anesthesia, Iteration, and Round for Evaporimeter

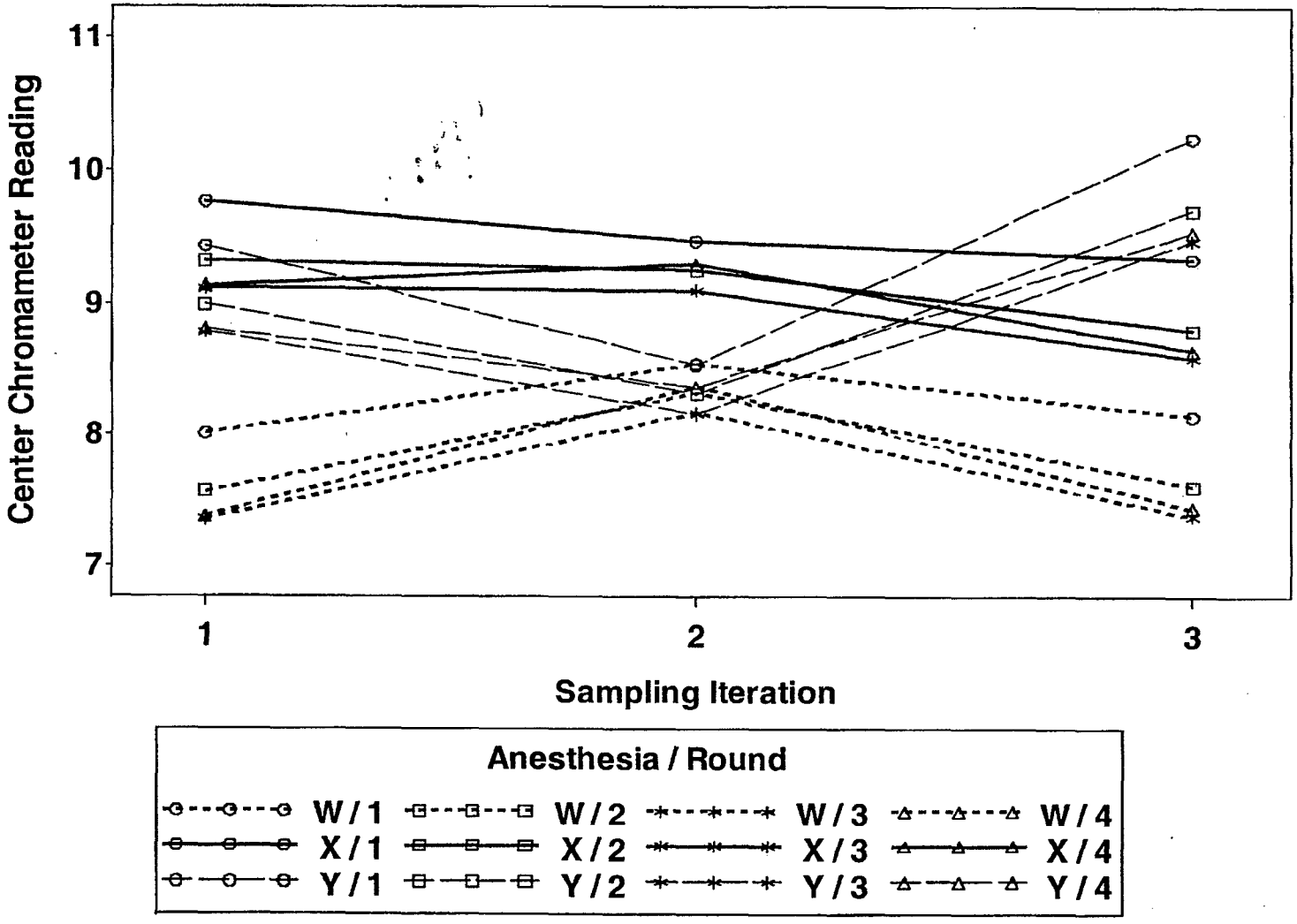

Figure 3. Estimated Response by Anesthesia, Iteration, and Round for Chromameter

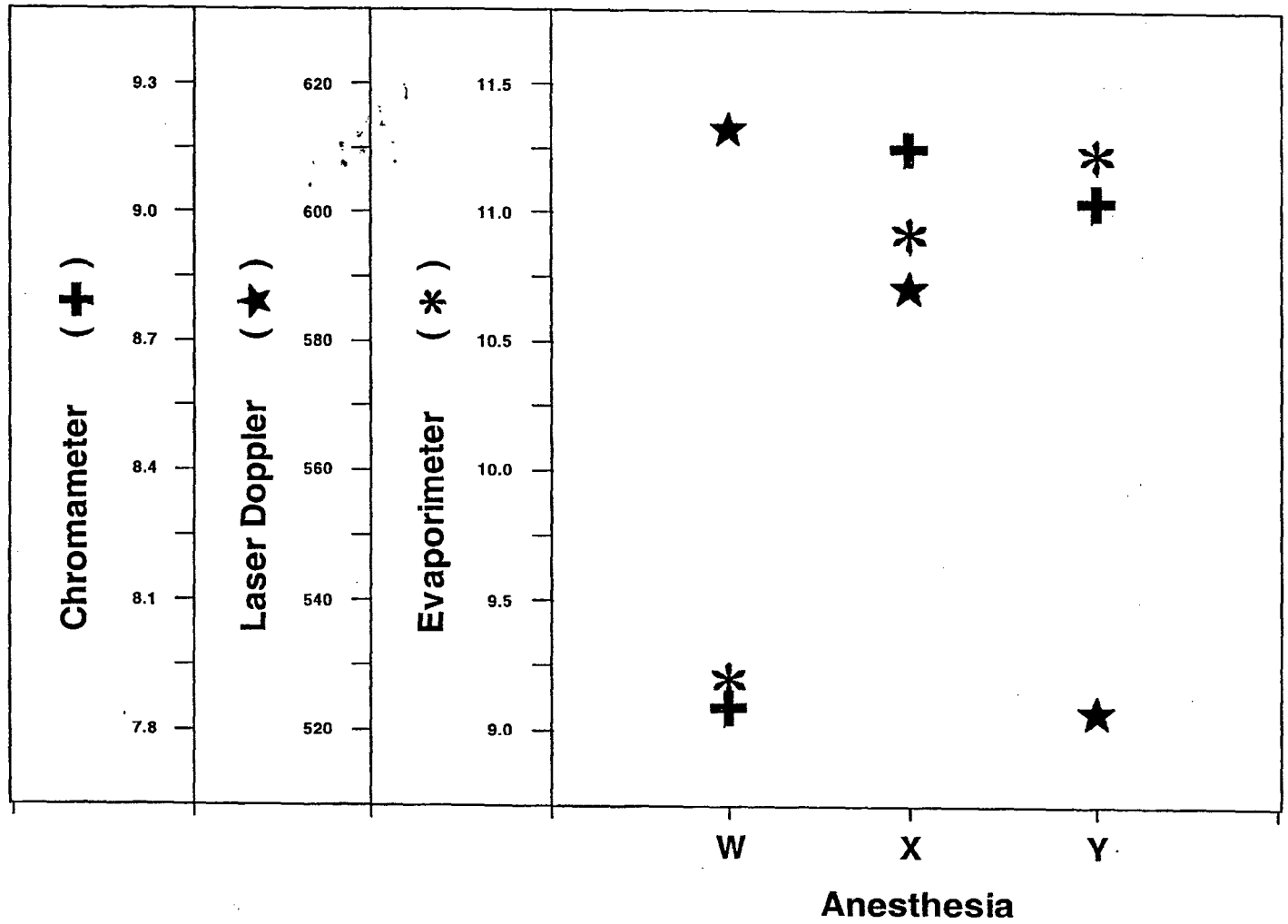

Figure 4. Estimated Response by Anesthesia for Chromameter, Laser Doppler, and Evaporimeter

FMR received 8-11-00

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Date August 10, 2000

To **Frances Reid**

From Nancy Niemuth/Shawn Shumaker

Subject **Statistical Report on MREF Task 94-33, Phase III, Part C - Revised**

s:\shum\Task 33\Phase IIIC\Phase IIIC-cvr memo-Revised + Phase IIIC-report-Revised.doc

The attached report summarizes the statistical analysis of high frequency ultrasound, laser Doppler perfusion imaging, reflectance colorimetry (Chromameter), evaporimeter, clinical observations, and histopathology data collected under MREF Task 94-33.

The report tables were revised at your request to identify biomechanical instrumentation, histopathology, and clinical observation parameters when these were included on the same table. In addition, the SE values reported in Table A-1 were corrected. In preparation of supporting documentation for QA, we noted that SDs had been reported for some parameters rather than SEs. No changes were made to the report text, as the revisions did not impact the methods, results, or conclusions previously reported. An electronic copy of this report will be provided for use in preparing the draft final report on this study.

Please call Nancy Niemuth at 4-3231, or Shawn Shumaker at 4-3232, if you have any questions on this report.

NAN/SMS:llj
Attachment

For Review and Approval

	Name	Initials	Date
Originator	Nancy Niemuth	NN	8/10/00
Originator	Shawn Shumaker	SMS	8/10/2000
Approved	Bill Rosebrough	WRR	8/16/2000

Sent Via: Interoffice Mail

MREF Task 94-33, Phase III, Part C. Statistical Report on Characterization of Full and Partial Thickness Wounds

August 10, 2000

Introduction

The purpose of MREF Task94-33, Phase III, Part C was to develop and characterize full-thickness and partial thickness dermal burns in weanling swine by changing exposure time using a 400 µl HD per site ventral abdominal application. A total of 19 animals were available for statistical analyses: 7 control, 6 full-thickness, and 6 partial-thickness animals. Six sites (A-F) on the ventral abdomen and two "offsite" control sites (C_1 and C_2) adjacent to sites C and D were available on each animal, as follows:

Anterior
A B
C_1 C D C_2
E F
Posterior

Sites A-F were exposed to HD or saline, according to group assignment of each animal. These were (1) a 30 minute exposure of 400 µl HD to produce a full-thickness wound, (2) a 2 minute exposure of 400 µl HD to produce a partial-thickness wound, or (3) saline control. Sites C_1 and C_2 were not exposed to HD or saline. Wounds were evaluated by: clinical observations, gross photography, histopathology, high frequency ultrasound, reflectance colorimetry (Minolta Chromameter), Laser Doppler perfusion imaging, and Evaporimeter readings. Each site was evaluated on study day 0 prior to exposure and again on study day 2. Tissue samples for histopathology were collected on study day 2. Replicate readings from the Chromameter and Evaporimeter instruments were averaged prior to statistical analysis.

Methods

Analysis of variance (ANOVA) models were fitted to the Ultrasound, Chromameter, Laser Doppler, and Evaporimeter data from control sites to determine (1) whether there was a positional effect and (2) whether there was a systemic effect due to HD-exposure. Additional ANOVA models were fitted to the data from sites A-F to assess the effects of HD-exposure using these instruments. The difference between baseline (day 0) and post-exposure (day 2) readings on each site was calculated as the endpoint for statistical analysis. No Ultrasound readings were available for animal 99-225-1 on day 0 and for sites C_1 and C_2 on animal 99-55-9 on day 2. Thus, the differences for these sites and animals could not be calculated for the analysis.

In all, three models were fitted to the readings from each instrument:

- **Model 1** included readings from sites A-F, C_1 and C_2 on control animals and sites C_1 and C_2 on full and partial thickness animals to test for positional effects. Model 1 was formulated as follows:

  (1) $\mathbf{Response_{ij}} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

  where $Response_{ij}$ = difference in instrument readings from day 0 to day 2 for the i^{th} site on the j^{th} animal
  μ = overall average value of the response
  α_i = effect of i^{th} site
  β_j = random effect of j^{th} animal
  ε_{ij} = random variation for the i^{th} site on the j^{th} animal

- **Model 2** included readings from C_1 and C_2 offsite control sites on all animals to test for systemic effects of HD-exposure on the offsite control sites. Model 2 was formulated as follows:

  (2) $\mathbf{Response_{ij}} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$

  where $Response_{ij}$ = difference in instrument readings from day 0 to day 2 for the i^{th} treatment on the j^{th} animal
  μ = overall average value of the response
  α_i = effect of i^{th} treatment
  β_j = random effect of j^{th} animal
  ε_{ij} = random variation for the i^{th} site on the j^{th} animal

- **Model 3** included readings from sites A-F on all animals to assess the effects of HD-exposure. Model 3 was formulated as follows:

  (3) $\mathbf{Response_{ijk}} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \delta_k + \varepsilon_{ijk}$

  where $Response_{ijk}$ = difference in instrument readings from day 0 to day 2 for the i^{th} site with j^{th} treatment on the k^{th} animal
  μ = overall average value of the response
  α_i = effect of i^{th} site
  β_j = effect of j^{th} treatment
  $\alpha\beta_{ij}$ = interaction effect between i^{th} site and j^{th} treatment
  δ_k = random effect of k^{th} animal
  ε_{ijk} = random variation for the i^{th} site with j^{th} treatment on the k^{th} animal

Each model was fitted using the SAS (V8) MIXED procedure. For Models 1 and 3, statistical contrasts of the model parameters were used to evaluate positional effects including front-to-back and onsite versus offsite (Model 1) positional effects. Bonferroni adjustments were made

to maintain an overall 0.05 level of significance over the multiple comparisons within each model. For Models 2 and 3, the Tukey-Kramer multiple comparisons procedure was used to evaluate treatment effects.

In addition, Model 3 was also fitted to the continuous burn depth variable in the histopathology data set, and the edema length, edema breadth, edema height, edema area, wound length, wound width, and wound area clinical observation variables. Models 1 and 2 could not be fitted for these endpoints, as all readings were zero at the control sites on both day 0 and day 2.

The rest of the histopathology and clinical observation data were categorical, of which there were two types: binary (0-1) variables indicating the absence or presence of a certain histopathologic or clinical observation endpoint, and endpoints scored on a 0 to 4 severity scale. Binary endpoints included the ulceration and hemorrhage histopathology data, and the clinical observation hemorrhage purple data. The histopathologic endpoints scored 0-4 were basal cell necrosis, depth of necrosis, and vascular necrosis, while the clinical observation endpoints were erythema extent, erythema description, edema, and necrosis.

For the binary histopathology and clinical observation data, Fisher's Exact tests were calculated to compare treatments. For the endpoints with severity scores, General ANOVA Scores tests were computed to compare treatments.

Finally, there were endpoints in both the histopathology and clinical observation data where all values were zero and could not be analyzed. These data consisted of the granulation and re-epithelization histopathologic endpoints, and the exudate, eschar, eschar scab percent covered, and infection clinical observation endpoints.

Results

Descriptive statistics for Ultrasound, Chromameter, Laser Doppler, Evaporimeter, and burn depth are provided in Table A-1 of appendix A, for each treatment group and site.

Tables 1 through 4 present model-predicted means, standard errors, and hypothesis test results for the Ultrasound, Chromameter, Laser Doppler, and Evaporimeter data.

Table 1 provides the results for Model 1 for all control sites from all animals. The third and fourth columns of this table contain the model-estimated means and SE for each of the continuous parameters. Almost all of the parameters showed a reduction between day 0 and day 2. This phenomenon is normal for control animals and was noted in phase III, part B anesthesia experiments. The last five columns contain the p-values for statistical comparisons to evaluate front-to-back and onsite vs. offsite positional effects. Only Laser Doppler showed evidence of a front-to-back positional effect between control animals. The model estimated mean decrease in Laser Doppler from day 0 to day 2 was 78.6 for sites A and B and 146.7 for sites E and F. As there were four contrasts performed for this model, p-values were compared at an alpha of 0.05/4=0.0125, using the Bonferroni adjustment for multiple comparisons.

Table 2 presents the results from Model 2 comparisons to determine whether there was a varying systemic effect of HD-exposure on the offsite control sites over the three treatment groups. The third and fourth columns of this table contain the model-estimated means and SE's for each of the four parameters. The last three columns contain the unadjusted p-values and the Tukey adjusted p-values for differences between treatments. When considering significance, an alpha of 0.05 was used to compare to the Tukey adjusted p-values. No significant differences were found, indicating no systemic effects due to treatments were seen for the offsite controls, C_1 and C_2.

Table 3 presents the results from the Model 3 evaluation of HD exposure effects on treated sites (A-F) on all animals. Ultrasound had a significantly greater change in the full group than in the control or partial group, which did not differ significantly from each other. Chromameter and Evaporimeter changes were significantly greater than the control group in both the partial and full groups. The mean changes did not differ between the partial and full groups for these instruments. In Laser Doppler there was a significantly greater change in the partial group than in the control or full group. The mean changes were not significantly different between the control and full group for Laser Doppler.

Burn depth was significantly different between the full and control group. Burn depths in the partial group were not significantly different from the control or from the full group. Edema length, breadth, height, and area were significantly different between the full and partial, and full and control groups, but the partial and control groups were not significantly different. Wound length, width, and area showed significant differences between the control and partial, and control and full groups. The partial and full groups were also significantly different for wound width and area, but not for wound length.

Table 4 presents the results from the Model 3 evaluation of positional effects in treated sites (A-F) on all animals, within each treatment group. For Ultrasound, changes at site CD were significantly greater than site AB in the full group. Chromameter changes were significantly greater for site EF than site AB in the partial group. Laser Doppler changes for site EF were significantly greater than those in site AB for the control, partial, and full groups. There were no significant positional effects for Evaporimeter.

There were not any significant positional effects within any of the treatment groups for burn depth, wound length, wound width and wound area. However, some significant results were seen in the full exposure group for the various edema endpoints. For edema area and breadth, site EF was significantly different from both site AB and CD, while edema height showed a significant difference between sites AB and CD, and edema length between sites AB and EF.

Categorical Data

Table 5 presents the counts and percentages of observations in each category for the histopathologic and clinical observation endpoints scored as present/absent, along with p-values from Fisher's Exact Tests comparing the three different treatment groups to each other. Results were considered significant when p-values were less than the Bonferroni adjusted alpha of 0.05/3 or 0.0167.

Table 6 presents the counts and percentages of animals in each class of the 0-4 severity scale and the results of the General ANOVA Scores test to compare the control, partial and full exposure treatment groups, for histopathologic and clinical observation endpoints that received severity scores.

Histopathologic endpoints:

As shown in Table 5, the incidence of hemorrhage in the control group was significantly less than the partial and full group incidences, while incidence of hemorrhage was not significantly different between the partial and full groups. Incidence of ulceration in the partial group was significantly greater compared to both the control and full groups, while the control and full group incidences were not significantly different from each other.

Severity scores for basal cell necrosis, depth of necrosis and vascular necrosis generally increased from the control to partial to full exposure groups, and all the treatment groups were significantly different from each other in all three endpoints (Table 6).

Clinical observation endpoints:

Incidence of hemorrhage purple in the full group was significantly greater than that in the control and partial groups. Hemorrhage purple was not observed in the control and partial groups and these groups were not significantly different (Table 5).

As shown in Table 6, severity scores for edema were significantly different between all of the groups. While severity scores for erythema description and erythema extent in the control group were significantly lower than those in the partial and full exposure groups, severity scores in the partial and full groups were not significantly different from each other for these two endpoints. Necrosis was significantly greater in the full exposure group than the control, at the Bonferroni adjusted alpha of $0.05/3 = 0.0167$, while the partial and full group comparison ($p=0.025$) was marginally significant based on the Bonferroni adjusted alpha. There was no evidence of necrosis in either the control or partial groups and these groups were not significantly different.

Conclusions

Although systemic effects due to HD-exposure were noted in earlier experiments conducted under MREF Task 94-33, no systemic effects of HD-exposure were noted on the offsite control sites using the Ultrasound, Chromameter, Laser Doppler, or Evaporimeter. In addition, no differences between the onsite and offsite control sites. Therefore, the use of within animal control sites for future experiments utilizing this model and these instruments is recommended.

There was mixed evidence that the control, partial and full groups were significantly different. All three groups were significantly different from each other for wound area, wound width, basal cell necrosis, depth of necrosis, vascular necrosis, and edema.

The full and partial thickness wounds were not significantly different for the following endpoints: Chromameter, Evaporimeter, burn depth, wound length, hemorrhage, erythema description, erythema extent, and necrosis.

In addition, the control and partial thickness wounds were not significantly different for the following endpoints: Ultrasound, burn depth, edema area, edema breadth, edema height, hemorrhage purple, and necrosis.

The control and full groups were not significantly different for Laser Doppler and ulceration.

Table 1. Evaluation of Positional Effects Using Control Sites on all Animals (Model 1)

Biomechanical Parameter	Site Groupings	Change From Day 0 to Day 2		P-Values for Comparison of Means Between Site Groupings				
		Mean	SE	AB	CD	EF	ABCDEF	C1 C2
Ultrasound	AB	-0.17	0.06		0.680	0.301		
	CD	-0.14	0.06	0.680		0.531		
	EF	-0.08	0.06	0.301	0.531			
	ABCDEF	-0.13	0.03					0.145
	C1 C2	-0.06	0.04				0.145	
Chromameter	AB	-0.12	0.36		0.340	0.371		
	CD	-0.44	0.36	0.340		0.953		
	EF	-0.42	0.36	0.371	0.953			
	ABCDEF	-0.33	0.30					0.200
	C1 C2	-0.66	0.26				0.200	
Laser Doppler	AB	-78.6	27.4		0.235	0.003 *		
	CD	-105.2	27.4	0.235		0.066		
	EF	-146.7	27.4	0.003 *	0.066			
	ABCDEF	-110.2	24.2					0.114
	C1 C2	-82.4	21.7				0.114	
Evaporimeter	AB	-3.53	1.13		0.798	0.389		
	CD	-3.25	1.13	0.798		0.265		
	EF	-4.51	1.13	0.389	0.265			
	ABCDEF	-3.76	0.93					0.665
	C1 C2	-3.40	0.79				0.665	

* indicates means are significantly different at an overall 0.05 level of significance when a Bonferroni adjustment for multiple comparisons is applied. Individual p-values were compared to 0.05/4=0.0125 for this comparison.

Table 2. Evaluation of Systemic Effects Due to HD Exposure in Offsite Control Sites on All Animals (Model 2)

Biomechanical Parameter	Treatment	Change From Day 0 to Day 2		P-Values for Comparison of Treatment Group Means (Unadjusted P-value / Tukey Adjusted P-value)		
		Mean	SE	Control[a]	Partial[b]	Full[c]
Ultrasound	Control[a]	0.06	0.08		0.066 / 0.151	0.221 / 0.430
	Partial[b]	-0.14	0.07	0.066 / 0.151		0.475 / 0.749
	Full[c]	-0.07	0.07	0.221 / 0.430	0.475 / 0.749	
Chromameter	Control[a]	-0.79	0.40		0.768 / 0.952	0.691 / 0.915
	Partial[b]	-0.61	0.43	0.768 / 0.952		0.921 / 0.994
	Full[c]	-0.55	0.43	0.691 / 0.915	0.921 / 0.994	
Laser Doppler	Control[a]	-68.2	27.7		0.359 / 0.622	0.873 / 0.986
	Partial[b]	-106.6	29.9	0.359 / 0.622		0.462 / 0.737
	Full[c]	-74.8	29.9	0.873 / 0.986	0.462 / 0.737	
Evaporimeter	Control[a]	-2.06	1.36		0.210 / 0.414	0.417 / 0.690
	Partial[b]	-4.65	1.47	0.210 / 0.414		0.658 / 0.895
	Full[c]	-3.72	1.47	0.417 / 0.690	0.658 / 0.895	

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

Table 3. Evaluation of HD Exposure Effects on Treated Sites (A-F) on all Animals (Model 3)

Parameter Type	Parameter	Treatment	Change From Day 0 to Day 2		P-Values for Comparison of Treatment Group Means (Unadjusted P-value / Tukey Adjusted P-value)		
			Mean	SE	Control[a]	Partial[b]	Full[c]
Biomechanical Instrumentation	Ultrasound	Control[a]	-0.13	0.20		0.163 / 0.341	<0.001 / <0.001 *
		Partial[b]	0.27	0.20	0.163 / 0.341		<0.001 / <0.001 *
		Full[c]	1.46	0.20	<0.001 / <0.001 *	<0.001 / <0.001 *	
	Chromameter	Control[a]	-0.42	0.58		<0.001 / <0.001 *	<0.001 / <0.001 *
		Partial[b]	8.40	0.63	<0.001 / <0.001 *		0.577 / 0.842
		Full[c]	8.89	0.63	<0.001 / <0.001 *	0.577 / 0.842	
	Laser Doppler	Control[a]	-98.7	50.2		<0.001 / 0.001 *	0.104 / 0.234
		Partial[b]	176.6	54.2	<0.001 / 0.001 *		<0.001 / <0.001 *
		Full[c]	-220.0	54.2	0.104 / 0.234	<0.001 / <0.001 *	
	Evaporimeter	Control[a]	-2.91	1.46		0.015 / 0.038 *	0.003 / 0.007 *
		Partial[b]	2.45	1.57	0.015 / 0.038 *		0.565 / 0.832
		Full[c]	3.73	1.57	0.003 / 0.007 *	0.565 / 0.832	
Histopathology[d]	Burn Depth, mm	Control[a]	0.00	0.27		0.060 / 0.142	0.001 / 0.003 *
		Partial[b]	0.76	0.29	0.060 / 0.142		0.150 / 0.319
		Full[c]	1.36	0.29	0.001 / 0.003 *	0.150 / 0.319	
Clinical Observations[d]	Edema Area, mm^2	Control[a]	-0.00	49.49		1.000 / 1.000	0.000 / 0.000 *
		Partial[b]	0.00	49.49	1.000 / 1.000		0.000 / 0.000 *
		Full[c]	842.91	49.49	0.000 / 0.000 *	0.000 / 0.000 *	
	Edema Breadth, mm	Control[a]	0.00	1.26		1.000 / 1.000	0.000 / 0.000 *
		Partial[b]	-0.00	1.26	1.000 / 1.000		0.000 / 0.000 *
		Full[c]	31.97	1.26	0.000 / 0.000 *	0.000 / 0.000 *	
	Edema Height, mm	Control[a]	0.00	0.50		1.000 / 1.000	0.000 / 0.000 *
		Partial[b]	0.00	0.50	1.000 / 1.000		0.000 / 0.000 *
		Full[c]	4.28	0.50	0.000 / 0.000 *	0.000 / 0.000 *	
	Edema Length, mm	Control[a]	-0.00	1.47		1.000 / 1.000	0.000 / 0.000 *
		Partial[b]	-0.00	1.47	1.000 / 1.000		0.000 / 0.000 *
		Full[c]	31.42	1.47	0.000 / 0.000 *	0.000 / 0.000 *	
	Wound Area, mm^2	Control[a]	0.00	31.66		0.000 / 0.000 *	0.000 / 0.000 *
		Partial[b]	743.86	31.66	0.000 / 0.000 *		0.019 / 0.050 *
		Full[c]	851.11	31.66	0.000 / 0.000 *	0.019 / 0.050 *	
	Wound Length, mm	Control[a]	0.00	0.65		0.000 / 0.000 *	0.000 / 0.000 *
		Partial[b]	30.58	0.65	0.000 / 0.000 *		0.205 / 0.412
		Full[c]	31.75	0.65	0.000 / 0.000 *	0.205 / 0.412	
	Wound Width, mm	Control[a]	-0.00	0.85		0.000 / 0.000 *	0.000 / 0.000 *
		Partial[b]	30.81	0.85	0.000 / 0.000 *		0.007 / 0.020 *
		Full[c]	34.11	0.85	0.000 / 0.000 *	0.007 / 0.020 *	

* indicates means are significantly different at an overall 0.05 level of significance when a Tukey adjustment for multiple comparisons is applied.

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

d For histopathology and clinical observation endpoints, all responses on day 0 were observed or assumed to be zero. Thus, the response may be interpreted as the change from day 0 to day 2, or simply as the response on day 2.

Table 4. Evaluation of Positional Effects in Treated Sites (A-F) on All Animals, within each Treatment Group (Model 3)

Parameter Type	Parameter	Treatment	Site Grouping	Change From Day 0 to Day 2		P-Values for Comparison of Means between Site Groupings within each Treatment Group		
				Mean	SE	AB	CD	EF
Biomechanical Instrumentation	Ultrasound	Control[a]	AB	-0.17	0.22		0.843	0.618
			CD	-0.14	0.22	0.843		0.764
			EF	-0.08	0.22	0.618	0.764	
		Partial[b]	AB	0.21	0.22		0.941	0.345
			CD	0.22	0.22	0.941		0.384
			EF	0.37	0.22	0.345	0.384	
		Full[c]	AB	1.23	0.22		0.016 *	0.151
			CD	1.67	0.22	0.016 *		0.309
			EF	1.49	0.22	0.151	0.309	
	Chromameter	Control[a]	AB	-0.21	0.68		0.589	0.612
			CD	-0.53	0.68	0.589		0.974
			EF	-0.51	0.68	0.612	0.974	
		Partial[b]	AB	7.45	0.73		0.120	0.006 *
			CD	8.46	0.73	0.120		0.206
			EF	9.28	0.73	0.006 *	0.206	
		Full[c]	AB	9.14	0.73		0.975	0.279
			CD	9.11	0.73	0.975		0.293
			EF	8.43	0.73	0.279	0.293	
	Laser Doppler	Control[a]	AB	-67.1	52.2		0.290	0.008 *
			CD	-93.7	52.2	0.290		0.100
			EF	-135.2	52.2	0.008 *	0.100	
		Partial[b]	AB	207.6	56.4		0.516	0.006 *
			CD	190.0	56.4	0.516		0.035
			EF	132.2	56.4	0.006 *	0.035	
		Full[c]	AB	-181.9	56.4		0.331	0.002 *
			CD	-208.3	56.4	0.331		0.025
			EF	-269.8	56.4	0.002 *	0.025	
	Evaporimeter	Control[a]	AB	-2.68	1.95		0.898	0.665
			CD	-2.40	1.95	0.898		0.575
			EF	-3.66	1.95	0.665	0.575	
		Partial[b]	AB	2.80	2.10		0.247	0.468
			CD	-0.02	2.10	0.247		0.061
			EF	4.56	2.10	0.468	0.061	
		Full[c]	AB	5.35	2.10		0.515	0.179
			CD	3.77	2.10	0.515		0.486
			EF	2.08	2.10	0.179	0.486	
Histopathology[d]	Burn Depth, mm	Control[a]	AB	0.00	0.29		1.000	1.000
			CD	0.00	0.29	1.000		1.000
			EF	0.00	0.29	1.000	1.000	
		Partial[b]	AB	0.75	0.31		0.806	0.952
			CD	0.80	0.31	0.806		0.760
			EF	0.74	0.31	0.952	0.760	
		Full[c]	AB	1.32	0.31		0.664	0.295
			CD	1.24	0.31	0.664		0.140
			EF	1.52	0.31	0.295	0.140	

Table 4. (Continued)

Parameter Type	Parameter	Treatment	Site Grouping	Change From Day 0 to Day 2		P-Values for Comparison of Means between Site Groupings within each Treatment Group		
				Mean	SE	AB	CD	EF
Clinical Observations[d]	Edema Area, mm^2	Control[a]	AB	-0.00	63.65		1.000	1.000
			CD	-0.00	63.65	1.000		1.000
			EF	-0.00	63.65	1.000	1.000	
		Partial[b]	AB	-0.00	63.65		1.000	1.000
			CD	-0.00	63.65	1.000		1.000
			EF	0.00	63.65	1.000	1.000	
		Full[c]	AB	716.81	63.65		0.451	0.000 *
			CD	769.30	63.65	0.451		0.000 *
			EF	1042.6	63.65	0.000 *	0.000 *	
	Edema Breadth, mm	Control[a]	AB	-0.00	1.93		1.000	1.000
			CD	-0.00	1.93	1.000		1.000
			EF	0.00	1.93	1.000	1.000	
		Partial[b]	AB	-0.00	1.93		1.000	1.000
			CD	-0.00	1.93	1.000		1.000
			EF	-0.00	1.93	1.000	1.000	
		Full[c]	AB	28.92	1.93		0.974	0.000 *
			CD	28.83	1.93	0.974		0.000 *
			EF	38.17	1.93	0.000 *	0.000 *	
	Edema Height, mm	Control[a]	AB	0.00	0.81		1.000	1.000
			CD	0.00	0.81	1.000		1.000
			EF	-0.00	0.81	1.000	1.000	
		Partial[b]	AB	-0.00	0.81		1.000	1.000
			CD	0.00	0.81	1.000		1.000
			EF	-0.00	0.81	1.000	1.000	
		Full[c]	AB	2.83	0.81		0.007 *	0.262
			CD	5.92	0.81	0.007 *		0.101
			EF	4.08	0.81	0.262	0.101	
	Edema Length, mm	Control[a]	AB	-0.00	1.78		1.000	1.000
			CD	-0.00	1.78	1.000		1.000
			EF	-0.00	1.78	1.000	1.000	
		Partial[b]	AB	-0.00	1.78		1.000	1.000
			CD	-0.00	1.78	1.000		1.000
			EF	-0.00	1.78	1.000	1.000	
		Full[c]	AB	28.00	1.78		0.045	0.000 *
			CD	31.50	1.78	0.045		0.062
			EF	34.75	1.78	0.000 *	0.062	
	Wound Area, mm^2	Control[a]	AB	0.00	38.07		1.000	1.000
			CD	0.00	38.07	1.000		1.000
			EF	0.00	38.07	1.000	1.000	
		Partial[b]	AB	729.11	38.07		0.164	0.845
			CD	780.56	38.07	0.164		0.113
			EF	721.91	38.07	0.845	0.113	
		Full[c]	AB	822.64	38.07		0.523	0.095
			CD	846.14	38.07	0.523		0.297
			EF	884.56	38.07	0.095	0.297	

Table 4. (Continued)

Parameter Type	Parameter	Treatment	Site Grouping	Change From Day 0 to Day 2		P-Values for Comparison of Means between Site Groupings within each Treatment Group		
				Mean	SE	AB	CD	EF
Clinical Observations[d]	Wound Length, mm	Control[a]	AB	0.00	0.82		1.000	1.000
			CD	0.00	0.82	1.000		1.000
			EF	0.00	0.82	1.000	1.000	
		Partial[b]	AB	30.58	0.82		0.449	0.449
			CD	31.25	0.82	0.449		0.132
			EF	29.92	0.82	0.449	0.132	
		Full[c]	AB	31.25	0.82		0.395	0.395
			CD	32.00	0.82	0.395		1.000
			EF	32.00	0.82	0.395	1.000	
	Wound Width, mm	Control[a]	AB	-0.00	0.99		1.000	1.000
			CD	-0.00	0.99	1.000		1.000
			EF	-0.00	0.99	1.000	1.000	
		Partial[b]	AB	30.33	0.99		0.198	0.781
			CD	31.50	0.99	0.198		0.310
			EF	30.58	0.99	0.781	0.310	
		Full[c]	AB	33.50	0.99		0.853	0.067
			CD	33.67	0.99	0.853		0.099
			EF	35.17	0.99	0.067	0.099	

* indicates means are significantly different at an overall 0.05 level of significance when a Bonferroni adjustment for multiple comparisons is applied. Individual p-values were compared to 0.05/3=0.0167 for this comparison.

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

d For histopathology and clinical observation endpoints, all responses on day 0 were observed or assumed to be zero. Thus, the response may be interpreted as the change from day 0 to day 2, or simply as the response on day 2.

Table 5. Results for Histopathology and Clinical Observations Data Scored as Present/Absent

Parameter Type	Parameter	Treatment	Number of Observations (%)		P-Value for Comparison of Treatment Group Incidence		
			Absent	Present	Control[a]	Partial[b]	Full[c]
Histopathology	Ulceration	Control[a]	42 (100)	0 (0)		0.001 *	0.462
		Partial[b]	27 (75)	9 (25)	0.001 *		0.014 *
		Full[c]	35 (97)	1 (3)	0.462	0.014 *	
	Hemorrhage	Control[a]	41 (98)	1 (2)		<0.001 *	<0.001 *
		Partial[b]	3 (8)	33 (92)	<0.001 *		0.239
		Full[c]	0 (0)	36 (100)	<0.001 *	0.239	
Clinical Observations	Hemorrhage Purple	Control[a]	42 (100)	0 (0)		1.000	<0.001 *
		Partial[b]	36 (100)	0 (0)	1.000		<0.001 *
		Full[c]	20 (56)	16 (44)	<0.001 *	<0.001 *	

* indicates Fisher's Exact 2-sided test p-value significant at a Bonferroni adjusted alpha of 0.05/3=0.0167.

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

Table 6. Results for Histopathology and Clinical Observations Data Scored on a Severity Scale

Parameter Type	Parameter	Treatment	Number of Observations per Score (%)					P-Value for Comparison of Treatment Group Scores		
			0	1	2	3	4	Control[a]	Partial[b]	Full[c]
Histopathology	Basal Cell Necrosis	Control[a]	41 (98)	1 (2)	0 (0)	0 (0)	0 (0)		<0.001 *	<0.001 *
		Partial[b]	0 (0)	10 (28)	24 (67)	2 (6)	0 (0)	<0.001 *		<0.001 *
		Full[c]	0 (0)	0 (0)	0 (0)	33 (92)	3 (8)	<0.001 *	<0.001 *	
	Depth of Necrosis	Control[a]	41 (98)	1 (2)	0 (0)	0 (0)	0 (0)		<0.001 *	<0.001 *
		Partial[b]	0 (0)	0 (0)	0 (0)	36 (100)	0 (0)	<0.001 *		<0.001 *
		Full[c]	0 (0)	0 (0)	0 (0)	5 (14)	31 (86)	<0.001 *	<0.001 *	
	Vascular Necrosis	Control[a]	42 (100)	0 (0)	0 (0)	0 (0)	0 (0)		0.001 *	<0.001 *
		Partial[b]	28 (78)	8 (22)	0 (0)	0 (0)	0 (0)	0.001 *		<0.001 *
		Full[c]	2 (6)	24 (67)	10 (28)	0 (0)	0 (0)	<0.001 *	<0.001 *	
Clinical Observations	Edema	Control[a]	42 (100)	0 (0)	0 (0)	0 (0)	0 (0)		0.003 *	<0.001 *
		Partial[b]	29 (81)	7 (19)	0 (0)	0 (0)	0 (0)	0.003 *		<0.001 *
		Full[c]	3 (8)	0 (0)	0 (0)	21 (58)	12 (33)	<0.001 *	<0.001 *	
	Erythema Description	Control[a]	42 (100)	0 (0)	0 (0)	0 (0)	NA		<0.001 *	<0.001 *
		Partial[b]	0 (0)	7 (19)	26 (72)	3 (8)	NA	<0.001 *		0.266
		Full[c]	0 (0)	4 (11)	26 (72)	6 (17)	NA	<0.001 *	0.266	
	Erythema Extent	Control[a]	42 (100)	0 (0)	0 (0)	NA	NA		<0.001 *	<0.001 *
		Partial[b]	0 (0)	27 (75)	9 (25)	NA	NA	<0.001 *		1.000
		Full[c]	0 (0)	28 (78)	8 (22)	NA	NA	<0.001 *	1.000	
	Necrosis	Control[a]	42 (100)	0 (0)	0 (0)	0 (0)	0 (0)		1.000	0.008 *
		Partial[b]	36 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1.000		0.025
		Full[c]	30 (83)	0 (0)	6 (17)	0 (0)	0 (0)	0.008 *	0.025	

* indicates Nonparametric scores exact test p-value significant at a Bonferroni adjusted alpha of 0.05/3=0.0167.

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

NA = Not applicable

Figure 1. Mean (SE) Change in Ultrasound Readings from Day 0 to Day 2, for Control, Partial Thickness, and Full Thickness Groups

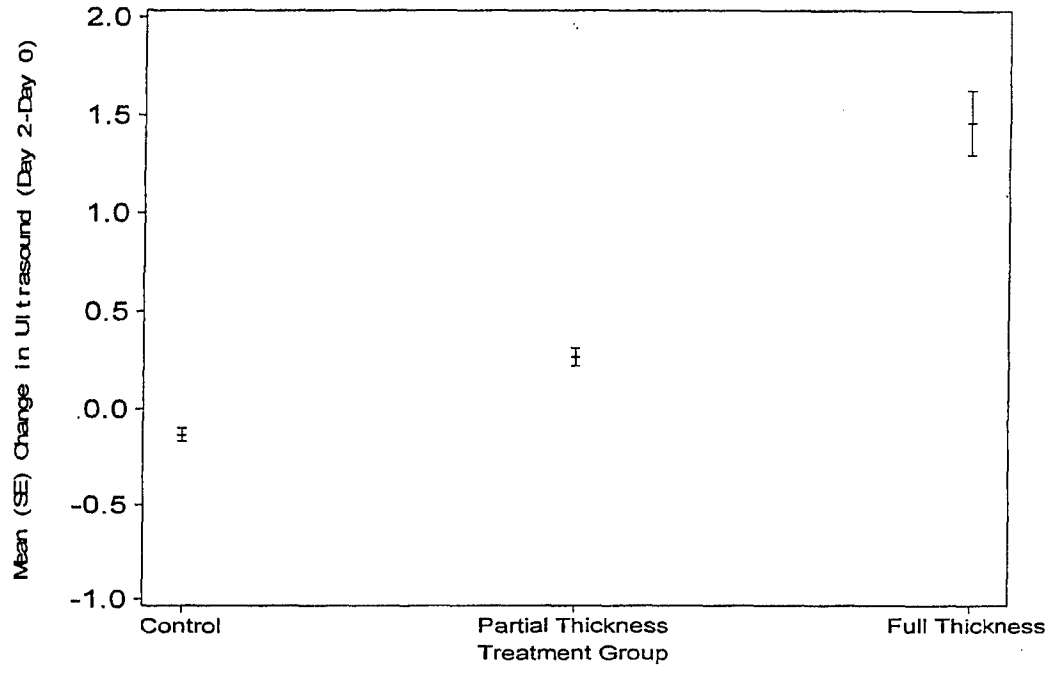

Figure 2. Mean (SE) Change in Chromameter Readings from Day 0 to Day 2, for Control, Partial Thickness, and Full Thickness Groups

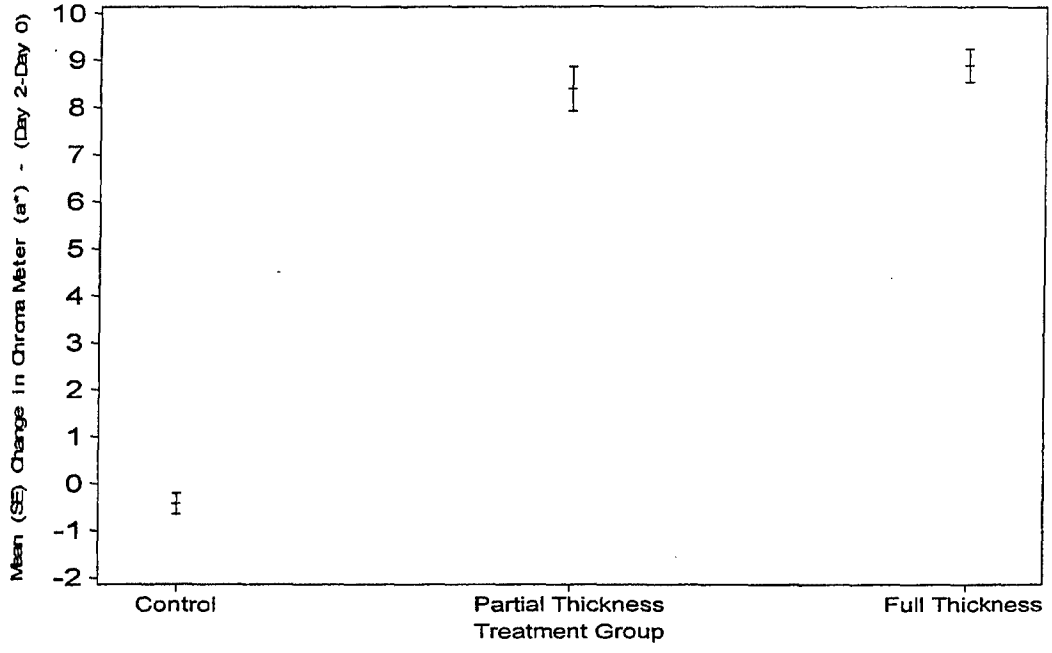

Figure 3. Mean (SE) Change in Laser Doppler Readings from Day 0 to Day 2, for Control, Partial Thickness, and Full Thickness Groups

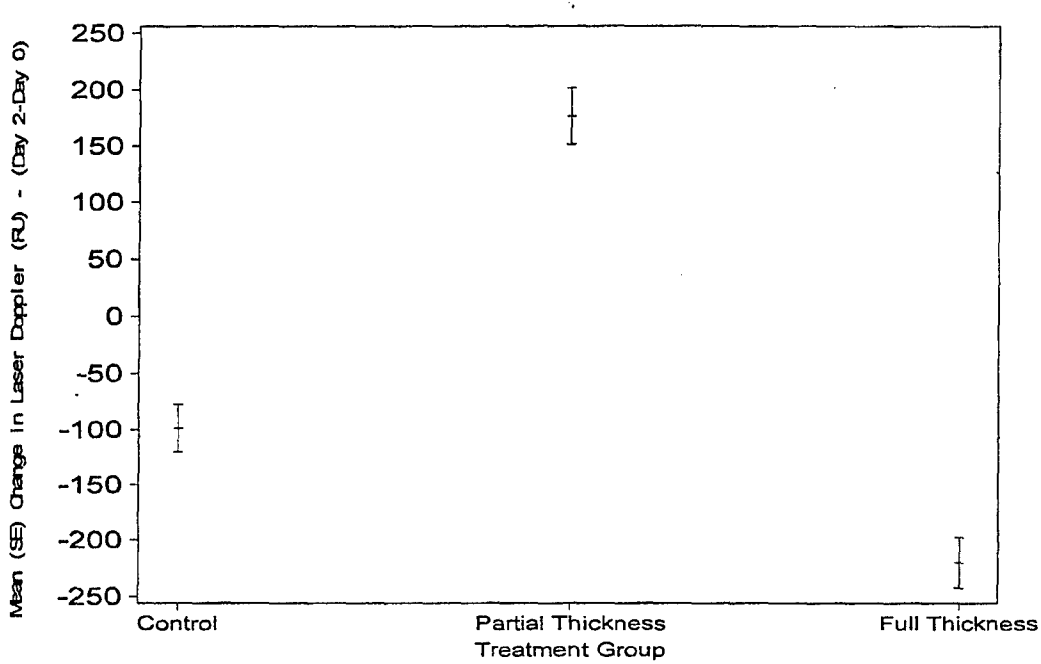

Figure 4. Mean (SE) Change in Evaporimeter Readings from Day 0 to Day 2, for Control, Partial Thickness, and Full Thickness Groups.

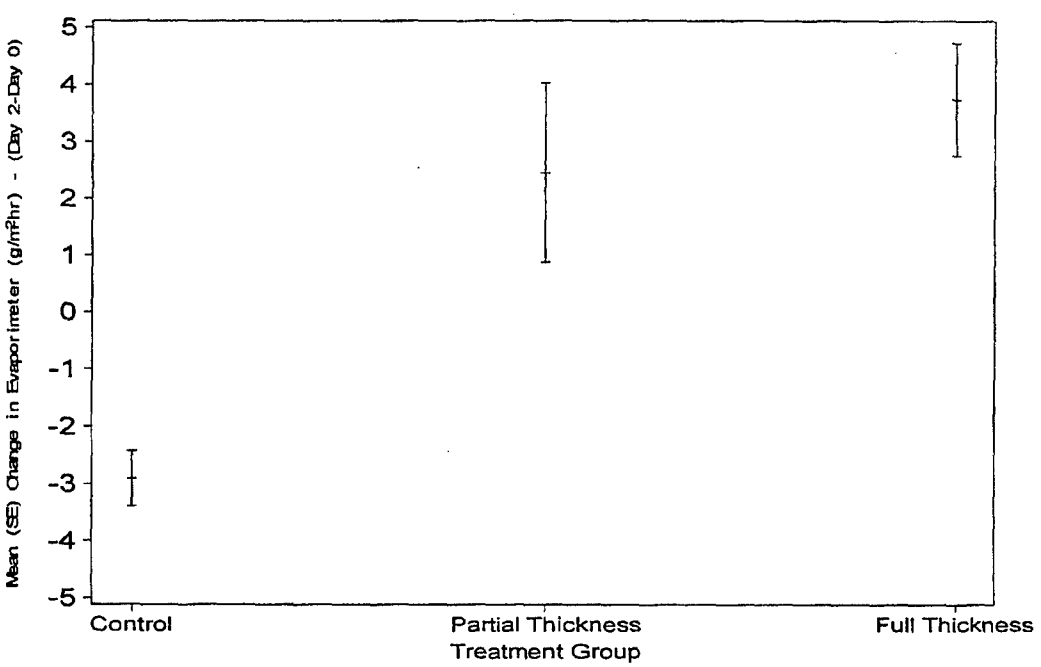

Table A-1. Descriptive Statistics for Ultrasound, Chromameter, Laser Doppler, Evaporimeter, and Burn Depth, by Treatment Group and Site

Parameter Type	Parameter	Treatment	Site Grouping	Change From Day 0 to Day 2		Site	Change From Day 0 to Day 2	
				Mean	SE		Mean	SE
Biomechanical Instrumentation	Ultrasound	Control[a]	A	-0.23	0.08	B	-0.11	0.07
			C	-0.06	0.08	D	-0.21	0.09
			E	-0.20	0.08	F	0.04	0.08
			C1	-0.01	0.15	C2	0.13	0.03
		Partial[b]	A	0.21	0.11	B	0.20	0.13
			C	0.34	0.11	D	0.10	0.07
			E	0.50	0.08	F	0.25	0.13
			C1	-0.08	0.10	C2	-0.20	0.03
		Full[c]	A	1.36	0.35	B	1.11	0.31
			C	1.44	0.37	D	1.90	0.59
			E	1.58	0.42	F	1.40	0.44
			C1	-0.05	0.10	C2	-0.09	0.08
	Chromameter	Control[a]	A	-0.48	0.45	B	0.06	0.54
			C	-0.24	0.34	D	-0.82	0.65
			E	-0.55	0.58	F	-0.48	0.78
			C1	-0.90	0.53	C2	-0.67	0.28
		Partial[b]	A	7.80	0.70	B	7.10	0.50
			C	9.59	1.60	D	7.33	0.98
			E	10.26	1.55	F	8.31	0.99
			C1	-0.45	0.33	C2	-0.77	0.57
		Full[c]	A	8.61	0.83	B	9.66	1.07
			C	8.46	0.55	D	9.77	0.72
			E	6.86	0.76	F	10.01	0.78
			C1	-0.42	0.52	C2	-0.68	0.61
	Laser Doppler	Control[a]	A	-71.5	48.3	B	-62.7	63.2
			C	-97.2	47.6	D	-90.3	41.7
			E	-152.6	55.0	F	-117.8	64.3
			C1	-62.4	23.6	C2	-74.0	26.5
		Partial[b]	A	184.6	46.2	B	230.6	58.2
			C	178.7	47.1	D	201.3	81.6
			E	183.2	65.6	F	81.3	74.0
			C1	-87.8	36.4	C2	-125.3	30.3
		Full[c]	A	-188.6	67.1	B	-175.2	44.7
			C	-231.0	64.4	D	-185.5	52.2
			E	-289.6	58.6	F	-250.0	50.7
			C1	-72.9	30.4	C2	-76.7	33.7

Table A-1. (Continued)

Parameter Type	Parameter	Treatment	Site Grouping	Change From Day 0 to Day 2		Site Grouping	Change From Day 0 to Day 2	
				Mean	SE		Mean	SE
Biomechanical Instrumentation	Evaporimeter	Control[a]	A	-3.01	1.27	B	-2.35	1.06
			C	-2.31	1.11	D	-2.48	0.87
			E	-3.11	0.95	F	-4.20	1.86
			C1	-1.98	1.25	C2	-2.13	1.14
		Partial[b]	A	-0.54	1.46	B	6.13	4.94
			C	0.52	3.43	D	-0.56	1.51
			E	1.13	2.16	F	7.99	6.71
			C1	-4.58	2.34	C2	-4.73	2.94
		Full[c]	A	5.50	2.37	B	5.20	2.63
			C	2.29	1.67	D	5.25	3.52
			E	2.06	2.38	F	2.09	2.14
			C1	-3.37	0.99	C2	-4.06	1.40
Histopathology[d]	Burn Depth, mm^2	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
			C1	0.00	0.00	C2	0.00	0.00
		Partial[b]	A	0.53	0.20	B	0.97	0.67
			C	0.92	0.48	D	0.67	0.43
			E	0.83	0.51	F	0.65	0.38
			C1	0.00	0.00	C2	0.00	0.00
		Full[c]	A	1.31	0.47	B	1.33	0.53
			C	1.36	0.39	D	1.12	0.18
			E	1.25	0.10	F	1.80	0.49
			C1	0.00	0.00	C2	0.00	0.00
Clinical Observations[d]	Edema Area, mm	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Full[c]	A	661.44	160.01	B	772.18	144.28
			C	772.57	158.84	D	766.03	183.28
			E	1042.88	69.65	F	1042.36	77.96
	Edema Breath, mm	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Full[c]	A	26.33	5.61	B	31.50	3.79
			C	29.00	5.77	D	28.67	6.18
			E	39.17	2.10	F	37.17	1.54

Table A-1. (Continued)

Parameter Type	Parameter	Treatment	Site Grouping	Change From Day 0 to Day 2		Site Grouping	Change From Day 0 to Day 2	
				Mean	SE		Mean	SE
Clinical Observations[d]	Edema Height, mm	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Full[c]	A	2.83	0.70	B	2.83	0.31
			C	9.17	4.63	D	2.67	0.56
			E	4.50	0.50	F	3.67	0.49
	Edema Length, mm	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Full[c]	A	26.17	5.54	B	29.83	2.85
			C	35.00	1.75	D	28.00	5.98
			E	33.83	1.19	F	35.67	2.04
	Wound Area, mm^2	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	761.58	32.70	B	696.65	37.53
			C	768.12	54.83	D	792.99	110.46
			E	706.99	62.38	F	736.84	52.40
		Full[c]	A	782.52	28.95	B	862.76	41.19
			C	859.75	48.12	D	832.52	40.57
			E	899.54	44.62	F	869.57	73.05
	Wound Length, mm	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	31.67	1.52	B	29.50	0.72
			C	32.33	1.41	D	30.17	1.80
			E	29.67	1.73	F	30.17	1.47
		Full[c]	A	30.83	0.70	B	31.67	0.76
			C	31.50	0.99	D	32.50	0.89
			E	32.17	1.01	F	31.83	1.40
	Wound Width, mm	Control[a]	A	0.00	0.00	B	0.00	0.00
			C	0.00	0.00	D	0.00	0.00
			E	0.00	0.00	F	0.00	0.00
		Partial[b]	A	30.67	0.42	B	30.00	1.15
			C	30.17	1.28	D	32.83	2.48
			E	30.17	1.30	F	31.00	1.06
		Full[c]	A	32.33	1.12	B	34.67	1.28
			C	34.67	1.17	D	32.67	1.56
			E	35.67	1.63	F	34.67	1.89

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

d For histopathology and clinical observation endpoints, all responses on day 0 were observed or assumed to be zero. Thus, the response may be interpreted as the change from day 0 to day 2, or simply as the response on day 2.

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Date November 2, 2000

To **Frances Reid**

From Shawn Shumaker

Subject **Additional Statistical Analyis for MREF Task 94-33, Phase III, Part C**

s:\shum\Task 33\Phase IIIC\Supplemental Report_Oct 2000\Phase IIIC-memo-alternative laser doppler.doc + Phase IIIC-report-alternative laser doppler.doc

The attached report summarizes additional analyses of the Laser Doppler test data collected in Phase III, Part C, of MREF Task 94-33. This report is issued as a supplement to the report entitled "Statistical Report on MREF Task 94-33, Phase III, Part C - Revised" dated August 10, 2000.

Laser Doppler readings on day 2 were normalized to offsite control readings for this analysis, as requested by the sponsor. Statistical models were fitted to the normalized data to examine treatment effects. Correlations between normalized Laser Doppler readings and histopathological measures of wound depth were also examined.

A copy of the file containing the report text and tables will be forwarded via electronic mail for use in preparing the final report on Task 94-33.

Please call me at 424-3232, or Nancy Niemuth at 424-3231, if you have any questions.

SMS/NAN:llj
Attachment

For Review and Approval

	Name	Initials	Date
Originator	Shawn Shumaker	SMS	11/02/2000
Concurrence	Nancy Niemuth	N	11/02/00
Approved	Ben Pierce	WRP	11/02/00

Sent via: Interoffice Mail + email

MREF Task 94-33, Phase III, Part C. Statistical Report on Characterization of Full and Partial Thickness Wounds Additional Analysis of Laser Doppler Data

November 2, 2000

Introduction

This report is issued as a supplement to the report entitled "Statistical Report on MREF Task 94-33, Phase III, Part C - Revised" dated August 10, 2000. The previous report included all analyses specified in the study protocol, with the exception of a correlation analysis comparing Laser Doppler readings to histopathological measures of wound depth. The present report includes the correlation analysis and additional analysis of Laser Doppler readings requested by the sponsor. The additional analysis considers an alternative Laser Doppler endpoint, calculated by normalizing the day 2 Laser Doppler readings on each animal to the day 2 offsite control readings on the same animal.

Methods

The normalized Laser Doppler reading was calculated for each site (A-F) on day 2, as the ratio of the reading for the site divided by the average of the two offsite control readings on the same animal. Three statistical models were described in the previous report. Of these, Models 1 and 3 were fitted to the normalized Laser Doppler readings on day 2. Model 2, which included only offsite control data, was not fitted as the offsite controls would have to be normalized to themselves. A statistical contrast within Model 1was used to demonstrate that the two offsite controls readings within each animal were not statistically different ($p=0.572$), so that the average may be used to normalize within animal.

Both Pearson's and Spearman's correlation coefficients were calculated to examine the relationship between normalized Laser Doppler Readings and wound depth (mm) and depth of necrosis (scored as 0-4). The SAS (V8) CORR procedure was used for this analysis. The full and partial groups were included in the analysis.

Results

Descriptive statistics for the normalized Laser Doppler readings are provided in Table A-1 of Appendix A, for each treatment group and site.

Model-predicted means, standard errors, and hypothesis test results for the normalized Laser Doppler data are presented in Tables 1 through 4. These tables correspond to Tables 1 to 4, respectively, in the previous report. Table 2, which summarizes the results from Model 2, is not included because Model 2 was not applicable to the normalized Laser Doppler readings evaluated in this report.

Table 1 provides the results for Model 1 for all control sites from all animals. The third and fourth columns of this table contain the model-estimated means and SE. The last five columns contain the p-values for statistical comparisons to evaluate front-to-back and onsite versus offsite positional effects. The normalized Laser Doppler readings showed evidence of an onsite-to-offsite positional effect in control animals. The model estimated mean normalized Laser Doppler reading for sites ABCDEF was 1.3 times greater than sites C1 and C2. As there were four contrasts performed for this model, p-values were compared at an alpha of 0.05/4=0.0125, using the Bonferroni adjustment for multiple comparisons.

Table 3 presents the results from the Model 3 evaluation of HD exposure effects on treated sites (A-F) on all animals. Mean normalized Laser Doppler readings were significantly different from each other for all treatment groups. Group means (SE) were 2.11 (0.08), 1.31 (0.07), and 0.96 (0.08) for the partial thickness, control, and full thickness groups, respectively.

Table 4 presents the results from the Model 3 evaluation of positional effects in treated sites (A-F) on all animals, within each treatment group. Mean normalized Laser Doppler readings decreased significantly from front (sites A and B) to middle (sites C and D) to back (sites E and F) in the partial group. In the full group, the mean for front sites was significantly greater than that of back sites. Although a similar trend was present, comparisons of middle sites to front and back were not statistically significant in the full group.

The correlation analysis suggests that normalized Laser Doppler readings on day 2 are significantly correlated with wound depth ($p=0.004$) and depth of necrosis ($p<0.001$). Results of the Pearson correlation analysis are reported for wound depth, while Spearman's is presented for depth of necrosis.

Conclusions

The mean normalized Laser Doppler readings from the three treatment groups were all significantly different from each other. The mean for the partial group was greater than those of the control and full groups. The control group mean was significantly greater than that of the full group. The same ordering of means was present in the previous analysis of differences between baseline and day 2 Laser Doppler readings, but the control group mean was not statistically significantly different from the full.

There was no evidence of a front to back positional effect in normalized Laser Doppler readings on day 2 in the onsite control sites. There was, however, a significant difference between onsite and offsite control sites. Mean normalized Laser Doppler readings decreased significantly from front to middle to back in the partial group. In the full group, the mean was significantly greater in the front sites than the back sites.

Table 1. Evaluation of Positional Effects Using Control Sites on all Animals (Model 1)

Biomechanical Parameter	Site Groupings	Ratio to Offsites		P-Values for Comparison of Means Between Site Groupings				
		Mean	SE	AB	CD	EF	ABCDEF	C1 C2
Laser Doppler, normalized to offsite controls on day 2	AB	1.30	0.04		0.683	0.919		
	CD	1.32	0.04	0.683		0.610		
	EF	1.30	0.04	0.919	0.610			
	ABCDEF	1.31	0.03					0.000 *
	C1 C2	1.00	0.03				0.000 *	

* indicates means are significantly different at an overall 0.05 level of significance when a Bonferroni adjustment for multiple comparisons is applied. Individual p-values were compared to 0.05/4=0.0125 for this comparison.

Table 2. ***Not Applicable to this Report.***

Table 3. Evaluation of HD Exposure Effects on Treated Sites (A-F) on all Animals (Model 3)

Biomechanical Parameter	Treatment	Ratio to Offsites		P-Values for Comparison of Treatment Group Means (Unadjusted P-value / Tukey Adjusted P-value)		
		Mean	SE	Control[a]	Partial[b]	Full[c]
Laser Doppler, normalized to offsite controls on day 2	Control[a]	1.31	0.07		0.000 / 0.000 *	0.002 / 0.005 *
	Partial[b]	2.11	0.08	0.000 / 0.000 *		0.000 / 0.000 *
	Full[c]	0.96	0.08	0.002 / 0.005 *	0.000 / 0.000 *	

* indicates means are significantly different at an overall 0.05 level of significance when a Tukey adjustment for multiple comparisons is applied.

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

Table 4. Evaluation of Positional Effects in Treated Sites (A-F) on All Animals, within each Treatment Group (Model 3)

Biomechanical Parameter	Treatment	Site Grouping	Ratio to Offsites		P-Values for Comparison of Means between Site Groupings within each Treatment Group		
			Mean	SE	AB	CD	EF
Laser Doppler, normalized to offsite controls on day 2	Control[a]	AB	1.30	0.08		0.758	0.938
		CD	1.32	0.08	0.758		0.700
		EF	1.30	0.08	0.938	0.700	
	Partial[b]	AB	2.28	0.09		0.016 *	0.000 *
		CD	2.11	0.09	0.016 *		0.010 *
		EF	1.93	0.09	0.000 *	0.010 *	
	Full[c]	AB	1.05	0.09		0.355	0.002 *
		CD	0.99	0.09	0.355		0.029
		EF	0.83	0.09	0.002 *	0.029	

* indicates means are significantly different at an overall 0.05 level of significance when a Bonferroni adjustment for multiple comparisons is applied. Individual p-values were compared to 0.05/3=0.0167 for this comparison.

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

Table A-1. Descriptive Statistics for Ultrasound, Chromameter, Laser Doppler, Evaporimeter, and Burn Depth, by Treatment Group and Site

Biomechanical Parameter	Treatment	Site Grouping	Ratio to Offsites		Site Grouping	Ratio to Offsites	
			Mean	SE		Mean	SE
Laser Doppler, normalized to offsite controls on day 2	Control[a]	A	1.24	0.08	B	1.36	0.06
		C	1.30	0.07	D	1.34	0.06
		E	1.33	0.09	F	1.26	0.08
		C1	0.99	0.03	C2	1.01	0.03
	Partial[b]	A	2.18	0.08	B	2.39	0.11
		C	2.05	0.13	D	2.17	0.17
		E	2.03	0.15	F	1.83	0.16
		C1	1.04	0.03	C2	0.96	0.03
	Full[c]	A	0.99	0.10	B	1.11	0.06
		C	0.94	0.11	D	1.03	0.09
		E	0.79	0.06	F	0.87	0.07
		C1	1.01	0.02	C2	0.99	0.02

a saline control

b 2-minute dermal exposure to 400 µl HD/site

c 30-minute dermal exposure to 400 µl HD/site

ATTACHMENT G

Chemistry Reports – All Phases

INTERNAL REPORT

from

Chemistry

on

Project Number G155533A

HD Stability in Liquids

to

Dr. Frances M. Reid
Project Director

March, 1999

Revised on September 28, 1999

by

Mr. Timothy L. Hayes
Dr. Theodore L. Miller

Battelle's Medical Research and
Evaluation Facility
505 King Avenue, JM-3
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M:/ Projects/Task 94-33/Chemistry/Reports/Internal Report.doc

Introduction

The chemistry group at Battelle's Medical Research and Evaluation Facility (MREF) was tasked by the Task 33 Study Director to evaluate the stability of HD in the following four liquids: peanut oil, propylene glycol, polyethylene glycol (PEG) 200 and PEG 400. This preliminary investigation was needed to assess the possibility of dosing HD mixtures over the 25 to 100 percent concentration range.

Experimental

The experimental design for this project followed typical analytical procedures used to establish the stability of chromatographable organic compounds in solution. Periodically, an aliquot of the solution was diluted (1:500) in methylene chloride for analysis. The mid-point concentration (50 percent HD) was selected to test the stability of each mixture. Because dosing solutions would typically be prepared fresh daily, the stability testing was only conducted at room temperature overnight. The four test liquids were purchased from J. T. Baker and HD was from Lot H12-1B.

To test the stability, a 50 percent (v/v) mixture of each test liquid was prepared with HD. An aliquot of each was immediately diluted in methylene chloride to measure the HD concentration at time zero. The two mixtures were stored at room temperature for about 19 hours. After this period, fresh dilutions were prepared from the stock material and analyzed

Prior to stability testing, the solubility of the test liquids were evaluated in GC solvents at the 1:500 level by adding 20 μL to the selected solvent in a 10 mL volumetric flask.

Samples were analyzed using a gas chromatograph (HP-5880A) equipped with a flame ionization detector (GC- FID). Samples were introduced using split injections via an autosampler and the sample components were separated on a Hewlett Packard HP-5 capillary column. A Hewlett Packard LAS Chromatography Data System was used for data acquisition. The instrumental parameters are listed in Table 1.

TABLE 1. GAS CHROMATOGRAPHY PARAMETERS

Gas Chromatograph:	Hewlett Packard 5880A
Data System:	Hewlett Packard LAS 3350
Autosampler:	Hewlett Packard 7672A
Analytical Column:	HP-5, 25 m x 0.32 mm ID x 0.52 μm film thickness
	Oven Conditions
Temperature Program:	50(0) to 215(0) @ 20C/min ; PT 300C(1)
Injector Temperature:	130° C
Detector Temperature:	300° C
	Injection Conditions
Injection Type:	Split using a 4 mm split liner with cup
Injection Volume:	1 μL
Split Flow:	85 mL/min

The linearity of the analysis method was determined by analyzing three calibration standards dispersed over the 0.186 to 1.50 mg/mL range using the GC conditions listed in Table 1. This range was chosen to provide sufficient quantitative data for the stability samples to below 25 percent of the starting concentration. The analytical standards were prepared from a HD stock solution. An example of the regression analysis values for triplicate injections using the simple linear model are presented in Table 2.

TABLE 2. LINEAR REGRESSION ANALYSIS RESULTS FOR CALIBRATION STANDARDS

Regression Output	
Constant	124.59
X Coefficient	245216
R Squared	0.9987

A three-point calibration curve was analyzed with each set of samples. The calibration data set is at a minimum comprised of two sets of three standards analyzed in triplicate. Peak area values were used to calculate the HD concentration from a simple linear regression model (y=mx+b).

Results and Discussion

Preliminary results showed that the liquids being evaluated were not very soluble in hexane but more soluble in chloroform. The solubility results indicated that methylene chloride would be the best solvent for both diluting the samples and the GC analysis. GC results of the four liquids by themselves showed that they would not interfere with the HD analysis via GC-FID. Figures 1 to 4 show chromatograms for each of the liquids. Although there are several components observed in these chromatograms, they are outside of the HD window at 5.65 min (see Figures 5 and 6). PEG 400 was not soluble in hexane and the least soluble in chloroform so it was not included in the subsequent miscibility and stability testing of HD.

Peanut oil and PEG 200 were found to be miscible with neat HD but propylene glycol was not. The propylene glycol and HD mixture was cloudy and then, separated into layers after a few minutes. Consequently, the propylene glycol HD test mixture could not be diluted for stability testing.

The results for the HD/Peanut oil and HD/PEG 200 mixtures are presented in Table 3. Chromatograms for the samples prepared after approximately 19 hr are shown in Figures 5 and 6.

TABLE 3. ANALYSIS RESULTS FOR STABILITY SAMPLES

Sample Description	Expected HD Conc.	Measured HD Conc.	Percent of Expected
50% HD/Peanut oil, Time 0, Injection 1	1.16 mg/ mL	1.17 mg/ mL	101 %
50% HD/Peanut oil, Time 0, Injection 2	1.16 mg/ mL	1.17 mg/ mL	101 %
50% HD/Peanut oil, Time 0, Injection 3	1.16 mg/ mL	1.15 mg/ mL	99 %
50% HD/PEG 200, Time 0, Injection 1	1.16 mg/ mL	1.17 mg/ mL	101 %
50% HD/PEG 200, Time 0, Injection 2	1.16 mg/ mL	1.15 mg/ mL	99 %
50% HD/PEG 200, Time 0, Injection 3	1.16 mg/ mL	1.18 mg/ mL	102 %
50% HD/Peanut oil, ~19 hr, Injection 1	1.16 mg/ mL	1.19 mg/ mL	103 %
50% HD/Peanut oil, ~19 hr, Injection 2	1.16 mg/ mL	1.15 mg/ mL	99%
50% HD/Peanut oil, ~19 hr, Injection 3	1.16 mg/ mL	1.16 mg/ mL	100 %
50% HD/PEG 200, ~19 hr, Injection 1	1.16 mg/ mL	1.15 mg/ mL	99%
50% HD/PEG 200, ~19 hr, Injection 2	1.16 mg/ mL	1.18 mg/ mL	102 %
50% HD/PEG 200, ~19 hr, Injection 3	1.16 mg/ mL	1.17 mg/ mL	101 %

CONCLUSION

The results shown in Table 3 demonstrate that HD/peanut oil and HD/PEG 200 mixtures at the 50 percent level are stable, within 3 percent of the expected HD concentration, for at least 19 hours when stored at room temperature. Proplyene glycol can not be used in HD dosing because it does not mix with HD. Since PEG 400 was not soluble in the GC solvent, it was not tested with HD.

FIGURE 1. TYPICAL CHROMATOGRAM FOR PEANUT OIL DILUTED 2.0 µL/mL HEXANE

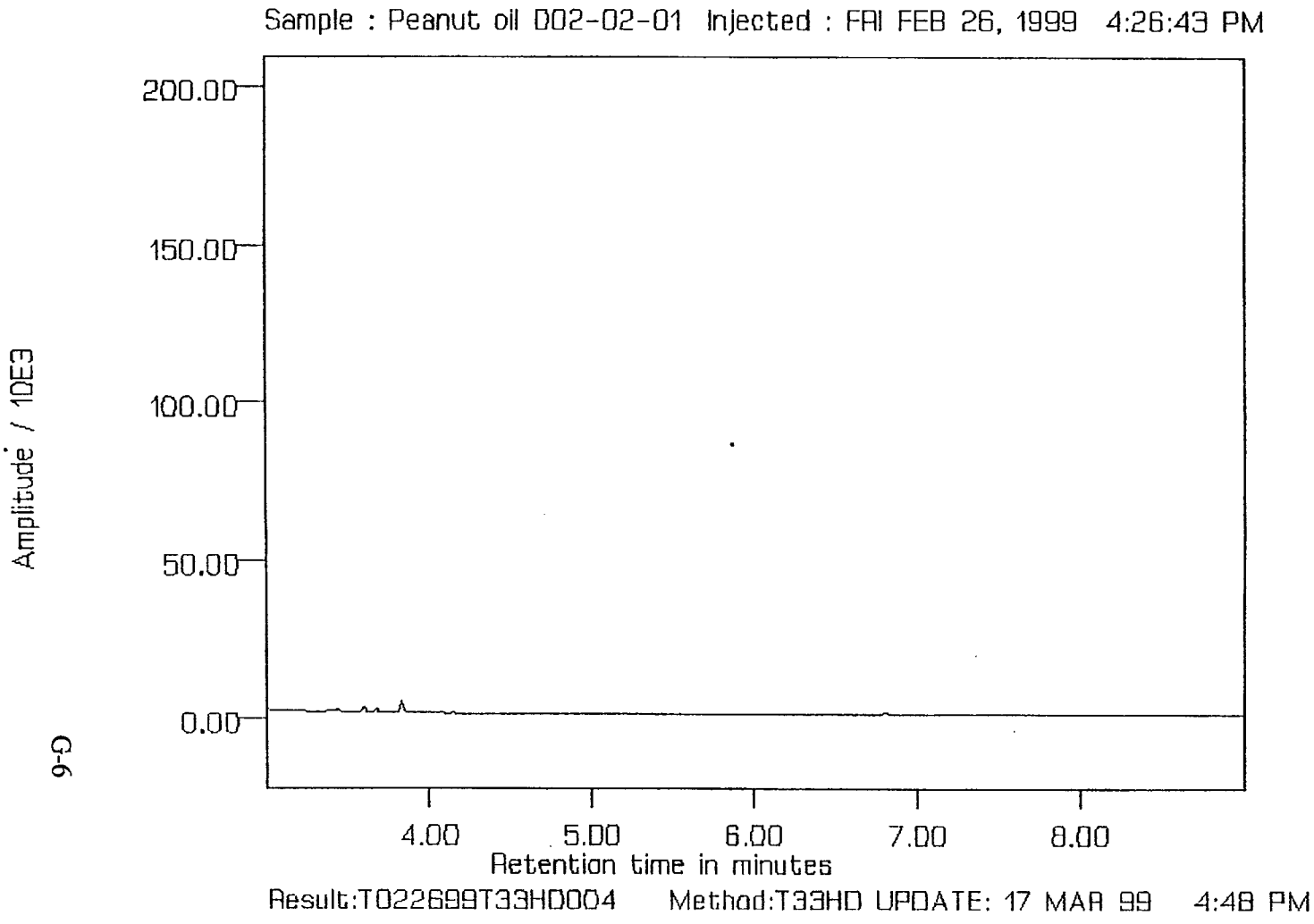


FIGURE 2. TYPICAL CHROMATOGRAM FOR FOR PROPYLENE GLYCOL DILUTED 2.0 µL/mL HEXANE

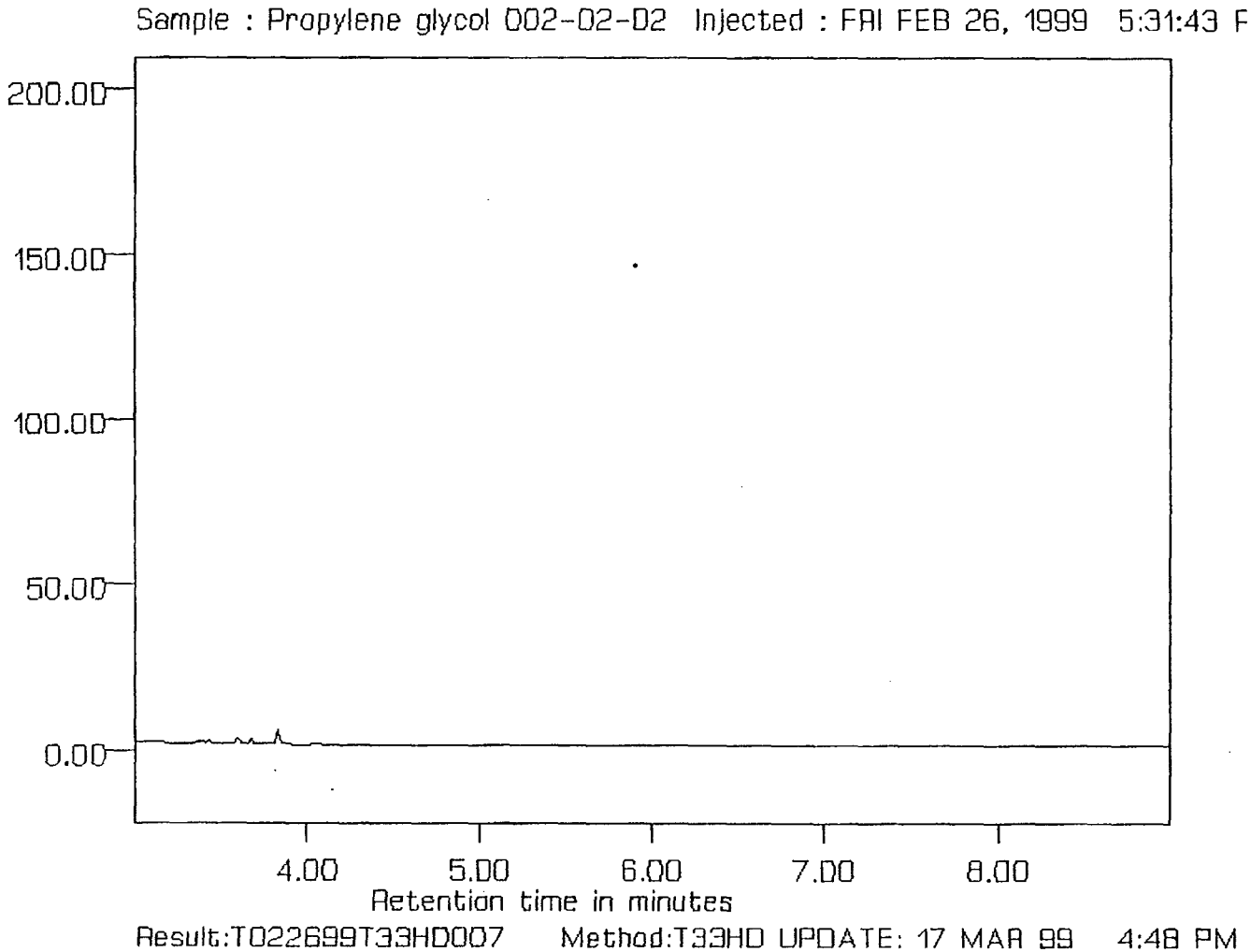


FIGURE 3. TYPICAL CHROMATOGRAM FOR FOR PEG 200 DILUTED 2.0 µL/mL HEXANE

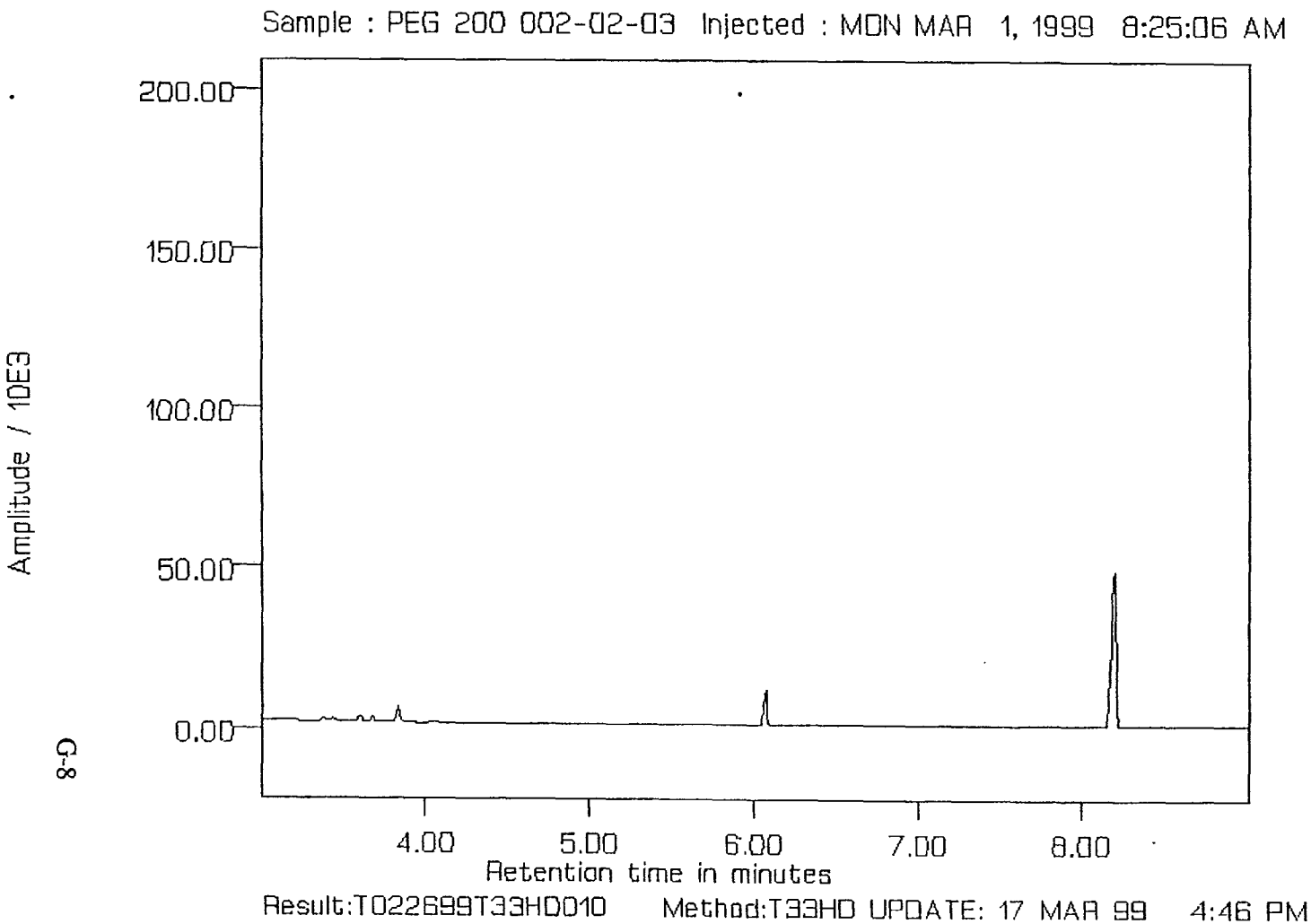


FIGURE 4. TYPICAL CHROMATOGRAM FOR FOR PEG 400 DILUTED 2.0 µL/mL CHLOROFORM

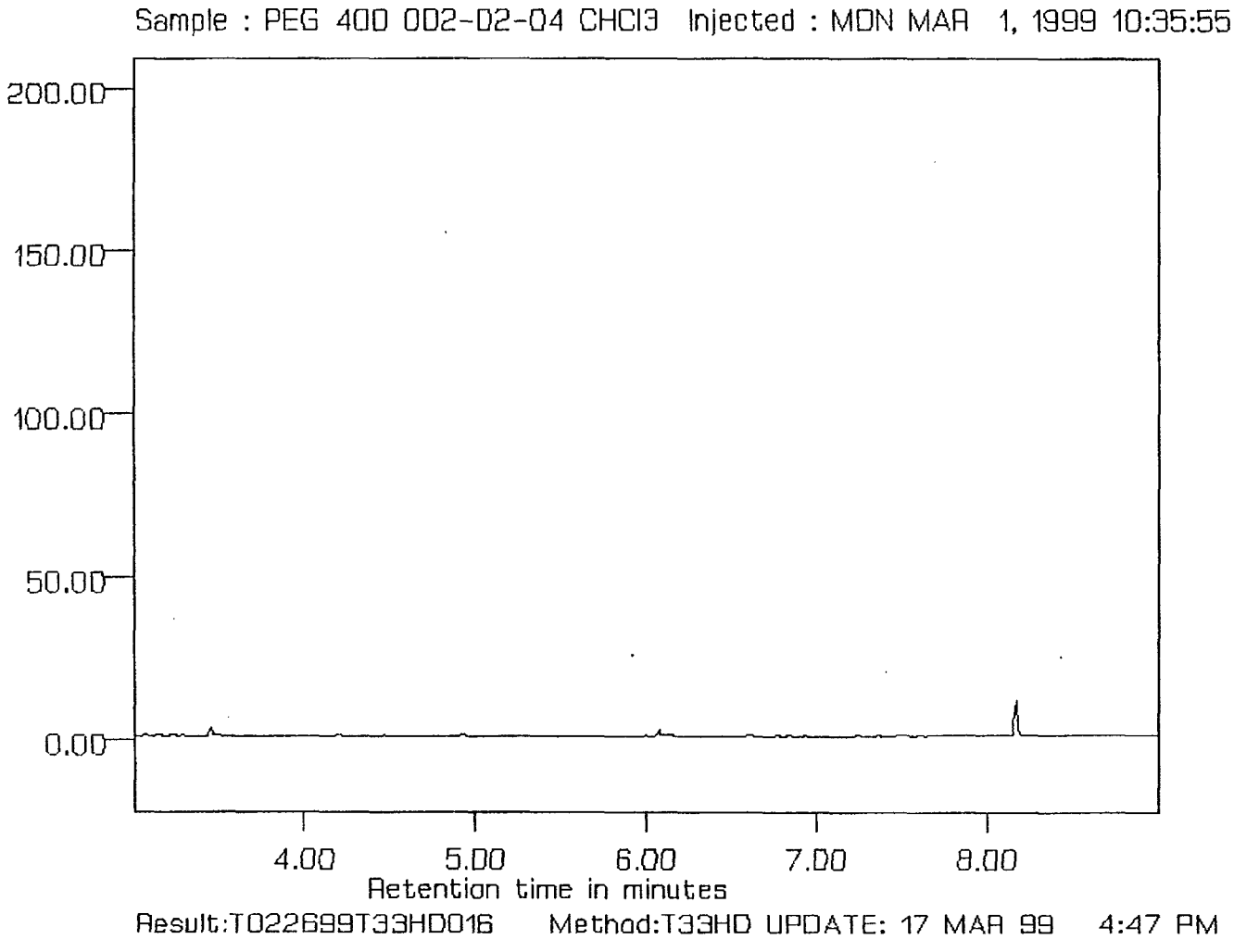


FIGURE 5. TYPICAL CHROMATOGRAM FOR A 50 PERCENT MIXTURE OF PEANUT OIL DILUTED TO 1.16 mg/mL HD IN METHYLENE CHLORIDE AFTER APPROXIMATELY 19 HOURS AT ROOM TEMP

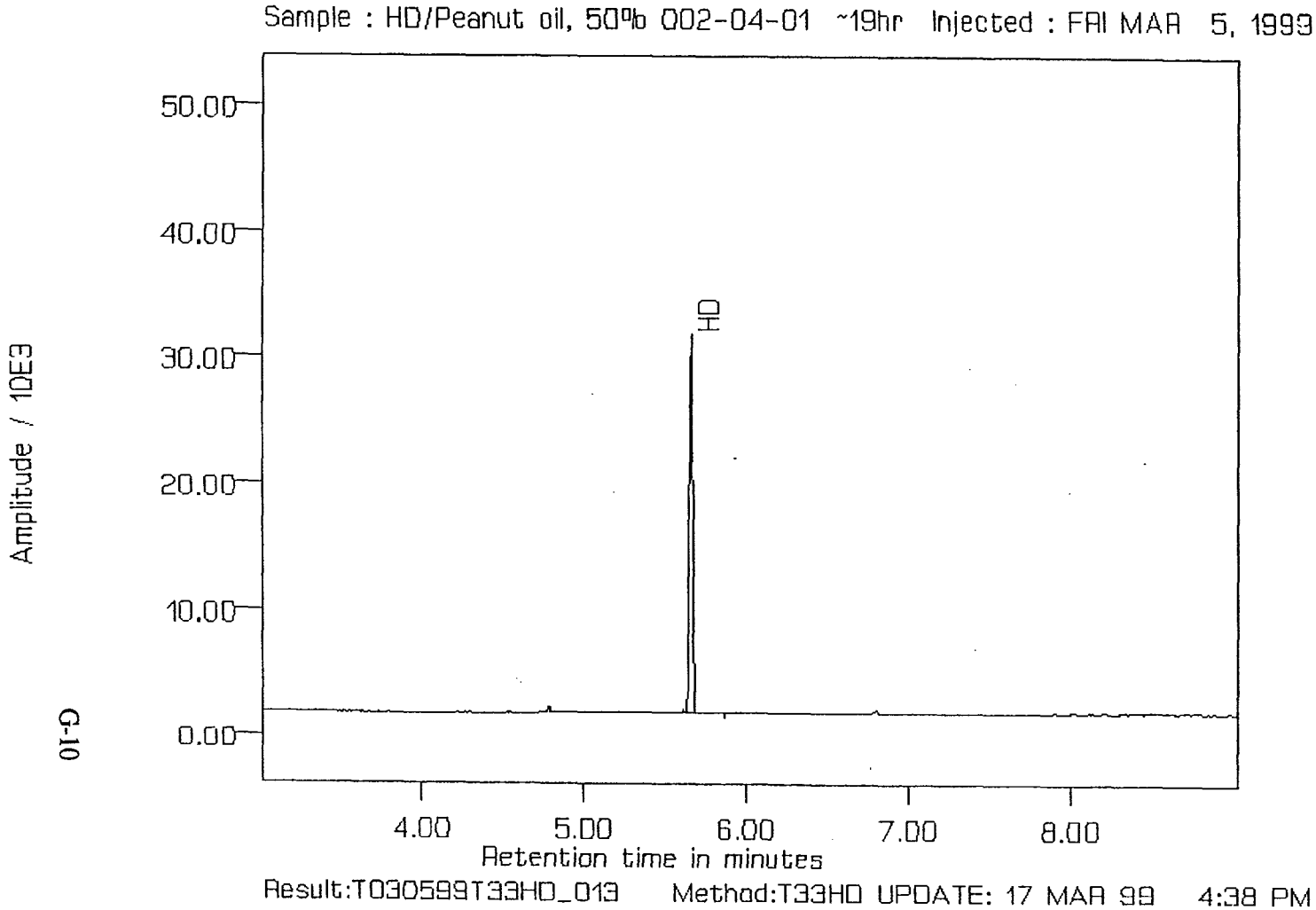
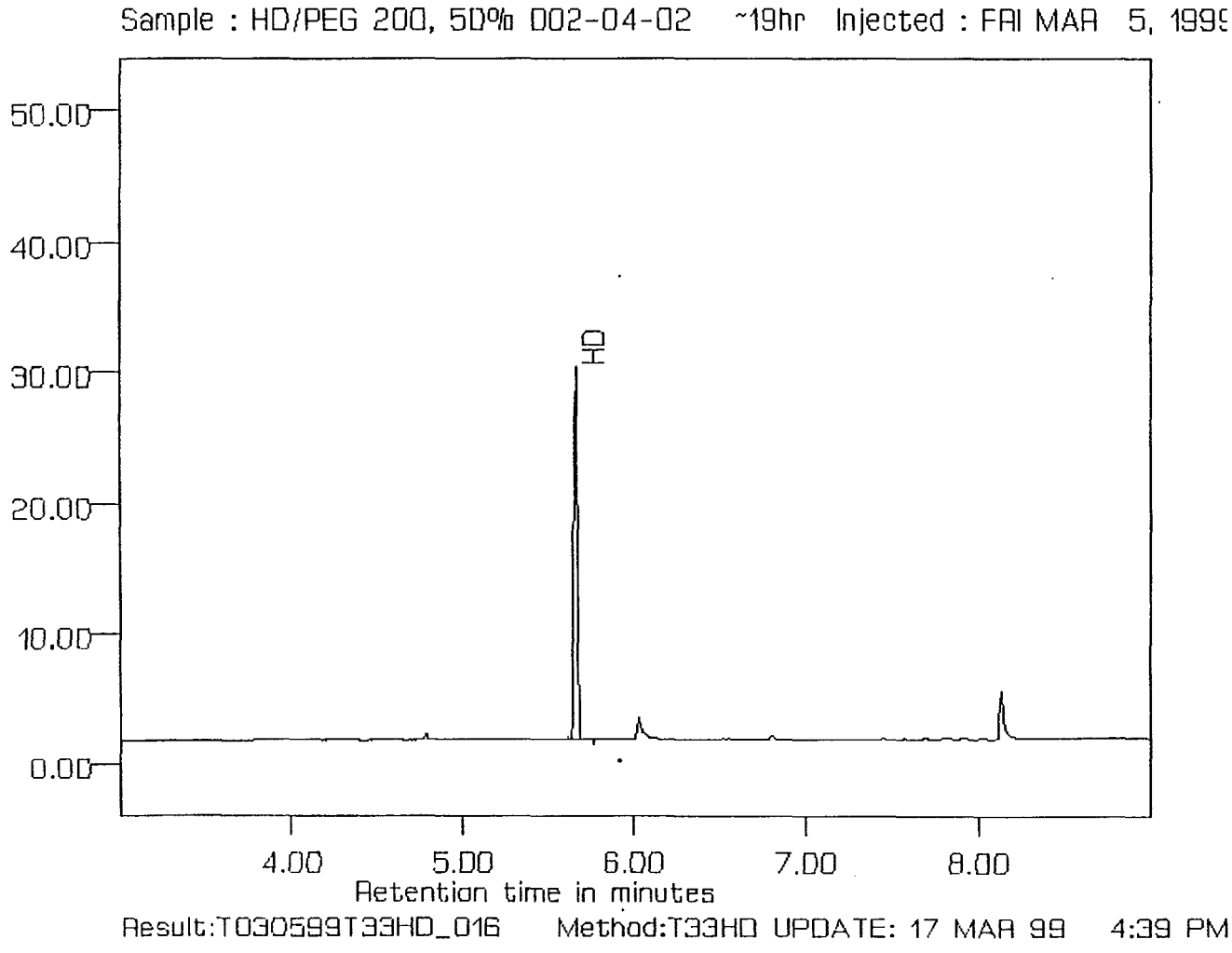


FIGURE 6. TYPICAL CHROMATOGRAM FOR A 50 PERCENT MIXTURE OF PEG 200 DILUTED TO 1.16 mg/mL HD IN METHYLENE CHLORIDE AFTER APPROXIMATELY 19 HOURS AT ROOM TEMPERATURE



INTERNAL REPORT

from

Chemistry

on

Project Number G155533A

Stability of Sulfur Mustard in Peanut Oil and PEG 200

to

Dr. Frances M. Reid

Study Director

July, 1999

Revised on September 28, 1999

by

Mr. Timothy L. Hayes TLH 8/10/00
Dr. Theodore L. Miller TLM 8-11-00

Battelle's Medical Research and
Evaluation Facility
505 King Avenue, JM-3
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Introduction

The chemistry group at Battelle's Medical Research and Evaluation Facility (MREF) was tasked by the Task 33 Study Director to evaluate the stability of sulfur mustard (HD) in the following four liquids: peanut oil, propylene glycol, polyethylene glycol (PEG) 200 and PEG 400. This investigation was needed to assess the feasibility of storing HD mixtures over the 25 to 75 percent concentration range in a freezer at approximately –70° C. During the preliminary testing phase, propylene glycol and PEG 400 were determined to be inappropriate as a dilutent. Consequently, they were eliminated from the study.[1]

This report will summarize the results for a thirty-five day stability study of HD in peanut oil and PEG 200 at the 25, 50 and 75 percent (v/v) levels stored in a freezer at approximately –70° C. The results for an eight-day stability test of all six mixtures stored at room temperature will also be presented.

Experimental

The experimental design for this project utilized procedures to establish the stability of chromatographable organic compounds in solution over time. Where periodically, an aliquot of each mixture was diluted in methylene chloride to yield solutions with about 1 mg/mL HD for analysis. The test liquids were purchased from J. T. Baker and the methylene chloride was from Burdick and Jackson. The HD was from MREF Lot H13-1A.

To test the stability 25, 50 and 75 percent (v/v) mixtures of peanut oil or PEG 200 were prepared with HD. The appropriate neat materials were delivered to a reaction vial with a gas tight syringe and thoroughly mixed by aspiration with a pipette. After mixing, an aliquot of each mixture was diluted in methylene chloride to measure the HD concentration at time zero. Then, the mixtures were aliquoted into GC vials. One set of mixtures was stored at room temperature for a single stability test on Day 8. All of the other GC vials were stored in a freezer at ≤ –70° C. Periodically over a period of 35 days, a set of mixtures was removed from the freezer for analysis. The mixtures were warmed to room temperature and diluted in methylene chloride for analysis. Dilutions of the set of mixtures stored at room temperature were only analyzed on the 8th day of the stability study.

Samples were analyzed using a gas chromatograph (GC; HP-5880A) equipped with a flame ionization detector (FID). Samples were introduced using split injections via an autosampler and the sample components were separated on a Hewlett Packard HP-5 capillary column. A Hewlett Packard LAS Chromatography Data System was used for data acquisition. The instrumental parameters are listed in Table 1.

TABLE 1. GAS CHROMATOGRAPHY PARAMETERS

Gas Chromatograph:	Hewlett Packard 5880A
Data System:	Hewlett Packard LAS 3350
Autosampler:	Hewlett Packard 7672A
Analytical Column:	HP-5, 25 m x 0.32 mm ID x 0.52 µm film thickness
	Oven Conditions
Temperature Program:	50(0) to 230(0) @ 20C/min ; PT 300C(15)
Injector Temperature:	160° C
Detector Temperature:	300° C
	Injection Conditions
Injection Type:	Split using a 4 mm split liner with cup
Injection Volume:	1 µL
Split Flow:	85 mL/min

The linearity of the analysis method was determined by analyzing four calibration standards dispersed over the 0.102 to 2.19 mg/mL range using the GC conditions listed in Table 1. This range was chosen to provide sufficient quantitative data for the stability samples to below 10 percent of the starting concentration. The analytical standards were prepared from a HD stock solution (Lot H13-1A). An example of the regression analysis values for triplicate injections using the simple linear model are presented in Table 2.

TABLE 2. LINEAR REGRESSION ANALYSIS RESULTS FOR CALIBRATION STANDARDS

Regression Output	
Constant	1760.1
X Coefficient	162669
R Squared	0.9998

A calibration curve was analyzed with each set of samples. The calibration data set was at a minimum comprised of two sets of four standards prepared at different analytical concentrations. Each standard was analyzed in triplicate and no more than five samples were analyzed between standards. Peak area values were used to calculate the HD concentration from a simple linear regression model (y=mx+b).

Results and Discussion

The results for the HD/peanut oil and HD/PEG 200 mixtures stored in a freezer at approximately –70° C are presented in Figures 1 to 6. The overall average values, standard deviations (STD) and relative standard deviations (RSTD) for the mixtures stored in a freezer at approximately –70° C are shown in Table 3. The average values from Table 3 are plotted in Figures 1 to 6 and the limits shown in these figures are based on the standard deviation values presented in Table 3 (Upper Limit = Average + 1 * STD; Lower Limit = Average - 1 * STD). The results for the eight-day stability test of mixtures stored at room temperature are given in Table 4.

FIGURE 1. Stability Results for the 25 Percent HD Mixture in Peanut Oil Stored in a Freezer at Approximately -70° C

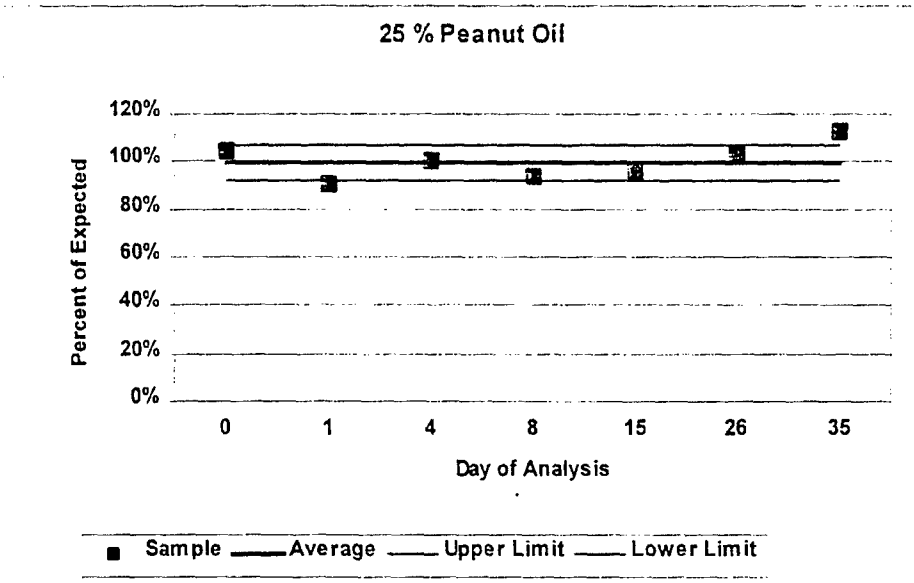

FIGURE 2. Stability Results for the 25 Percent HD Mixture in PEG 200 Stored in a Freezer at Approximately -70° C

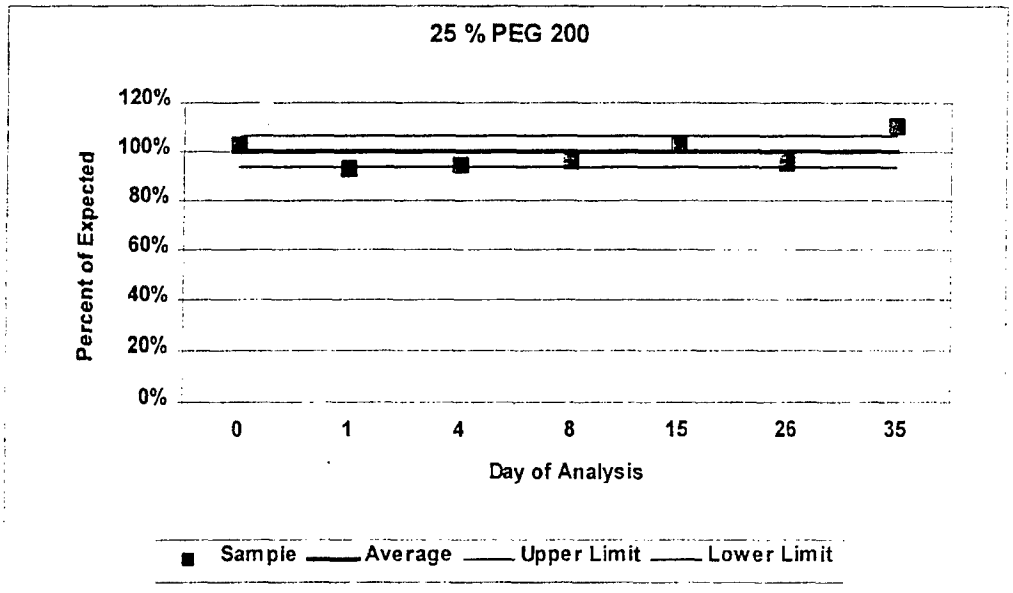

FIGURE 3. Stability Results for the 50 Percent HD Mixture in Peanut Oil Stored in a Freezer at Approximately -70° C

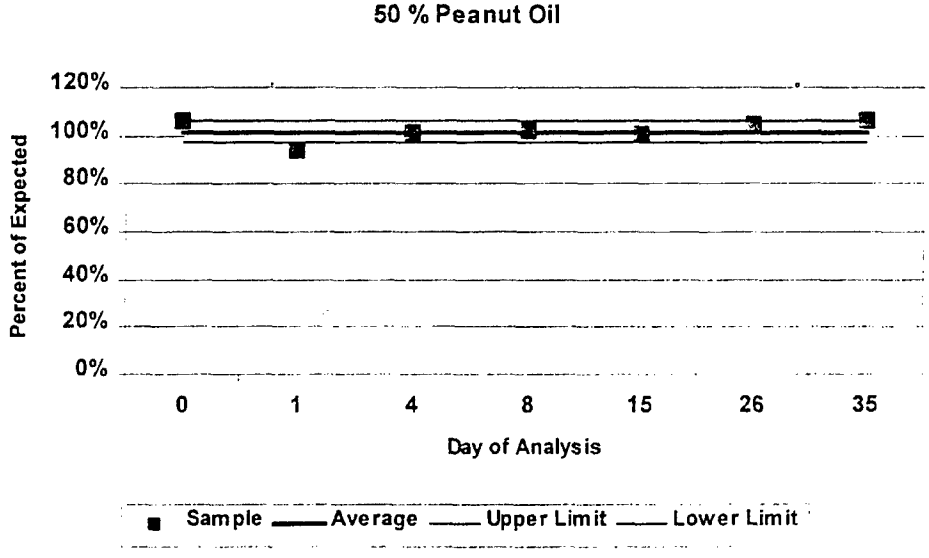

FIGURE 2. Stability Results for the 25 Percent HD Mixture in PEG 200 Stored in a Freezer at Approximately -70° C

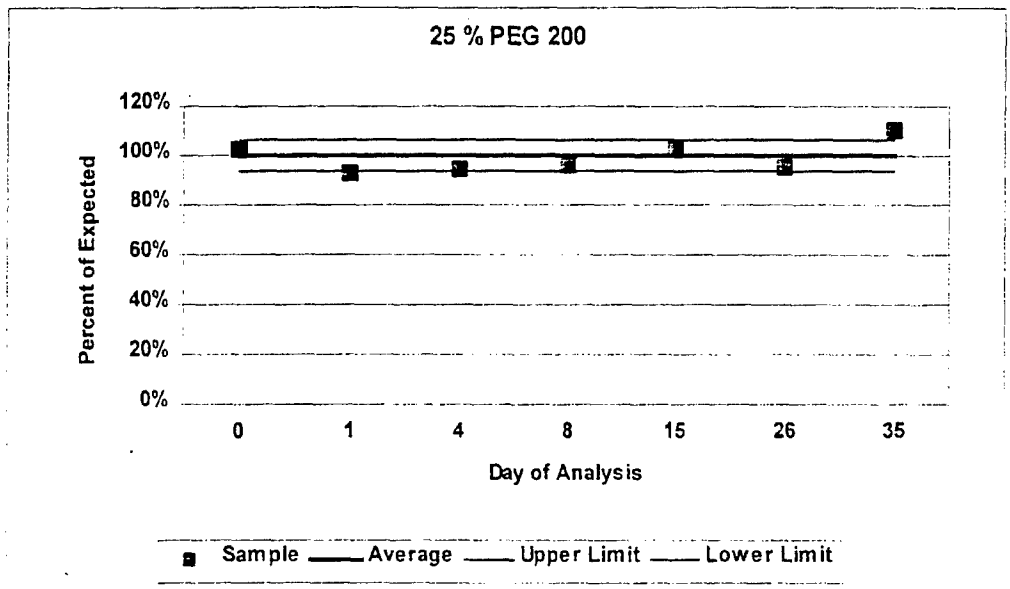

FIGURE 3. Stability Results for the 50 Percent HD Mixture in Peanut Oil Stored in a Freezer at Approximately -70° C

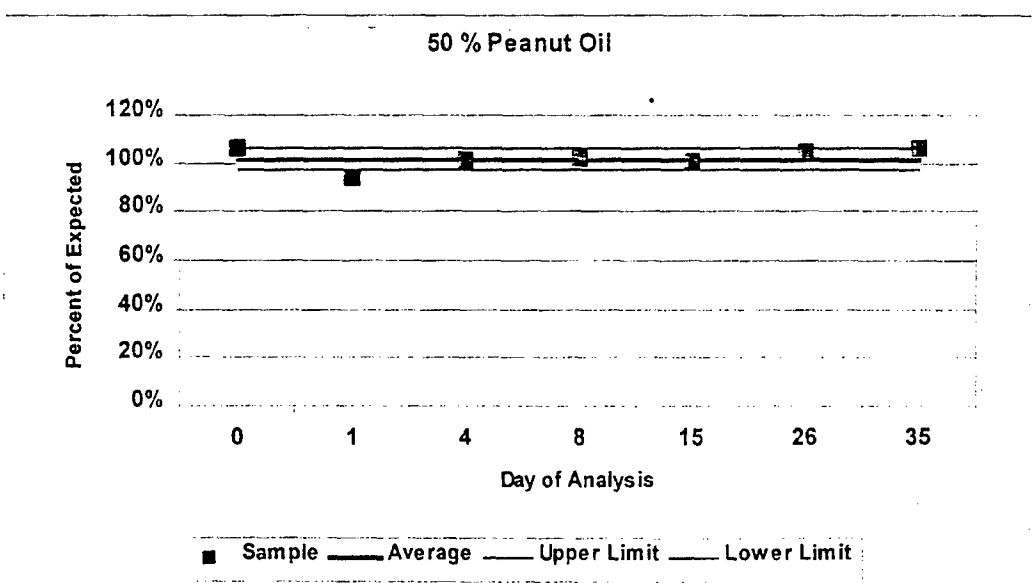

FIGURE 4. Stability Results for the 50 Percent HD Mixture in PEG 200 Stored in a Freezer at Approximately -70° C

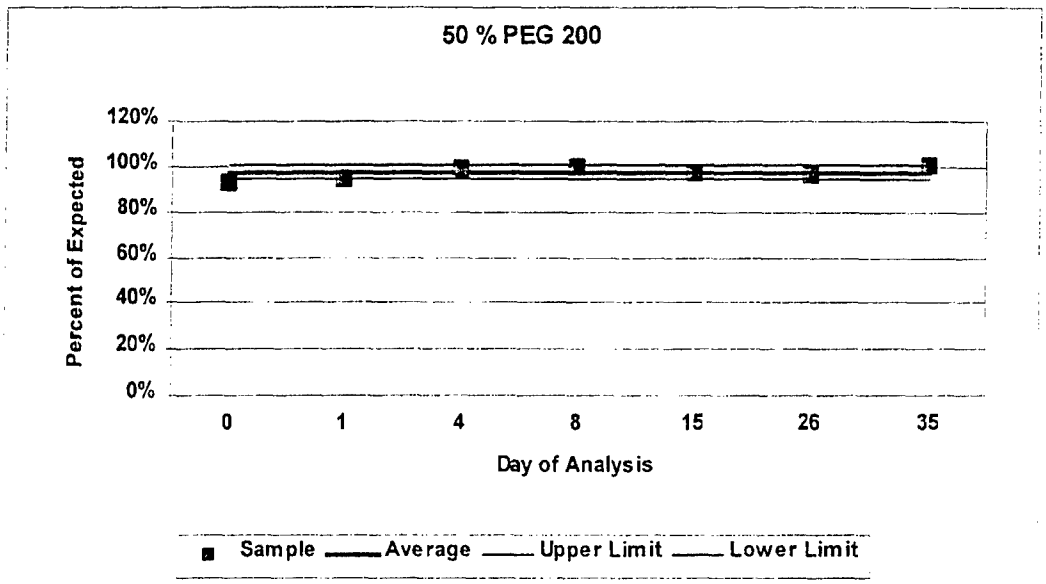

FIGURE 5. Stability Results for the 75 Percent HD Mixture in Peanut Oil Stored in a Freezer at Approximately -70° C

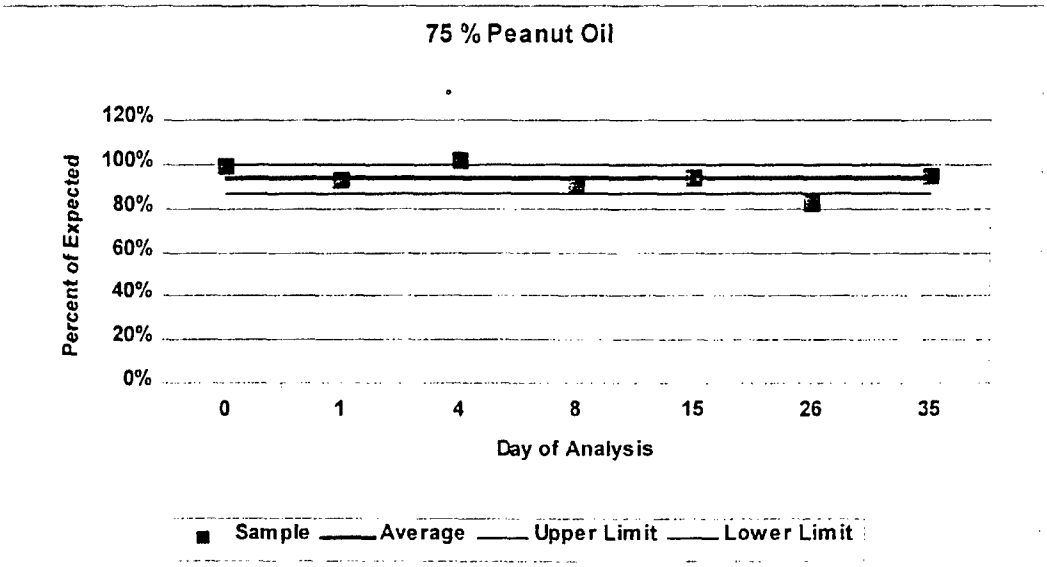

FIGURE 6. Stability Results for the 75 Percent HD Mixture in PEG 200 Stored in a Freezer at Approximately -70° C

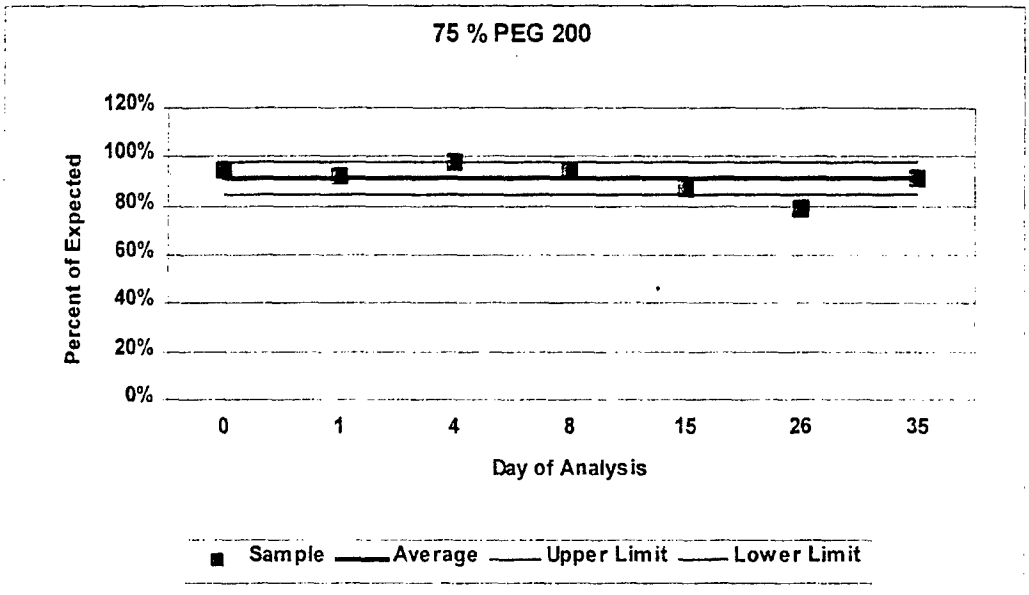

TABLE 3. STATISTICAL RESULTS FOR THE STABILITY MIXTURES STORED IN A FREEZER AT APPROXIMATELY –70° C

Sample	Average Percent of Expected	Standard Deviation	RSTD in Percent
25% Peanut Oil	99%	0.073	7.38%
25% PEG 200	100%	0.065	6.53%
50% Peanut Oil	102%	0.043	4.20%
50% PEG 200	97%	0.027	2.78%
75% Peanut Oil	93%	0.057	6.06%
75% PEG 200	91%	0.057	6.29%

TABLE 4. ANALYSIS RESULTS FOR THE STABILITY MIXTURES STORED AT ROOM TEMPERATURE

Sample	Average Percent of Expected
25% Peanut Oil	93%
25% PEG 200	108%
50% Peanut Oil	98%
50% PEG 200	99%
75% Peanut Oil	94%
75% PEG 200	92%

CONCLUSION

The results show that the HD/peanut oil and HD/PEG 200 mixtures at the 25, 50 and 75 percent levels are stable for more than 8 days when stored at room temperature and more than 35 days when stored in a freezer at approximately -70° C.

REFERENCES

1. Hayes, T. L. and T. L. Miller, March 1999. HD Stability in Liquids. Internal Battelle Report submitted to the Study Director.

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Project Number G155533A

Internal Distribution

T L Hayes
TL Miller
Jack Waugh
Task 94-33 File

Date September 29, 1999

To Dr. Frances M. Reid, Study Director

From Tim Hayes and Ted Miller

Subject Task 33 Completion: Stability of HD Mixtures, Dose Conformation and Pig POD

The studies that were conducted to evaluate the stability of HD in mixtures have been summarized in two reports submitted to the Study Director in March and July of this year. One error was correct on September 28, 1999 in the experimental section of both reports. The samples were actually analyzed on an HP 5890A instrument and not on a 5880A as reported. Both reports have been revised and copies of the revised reports have replaced the initial reports. These revised reports can be accessed electronically at the following location on the MREF M-drive: M:/Projects/Task 94-33/Chemistry/Reports/Internal Report.doc and M:/Projects/Task 94-33/Chemistry/Reports/Second Internal Report.doc. Data packages for the stability studies are located in Task 33 MREF Chemistry Binder 2 and the Lab Record Book (CLRB002) used for the stability investigation is located in Task 33 MREF Chemistry Binder 3.

Dose conformation samples were analyzed via GC-FPD like the stability samples except the GC injector temperature was 250° C instead of the lower temperature used for the stability samples containing either peanut oil or PEG. The other instrumental parameters used for dose conformation samples are listed in Table 1 of the stability reports cited above. The linearity of the analysis method was determined by analyzing at least four calibration standards. All of the measured HD concentration values for the dose conformation samples were within 10 percent of the expected value except for four samples that were analyzed in February and March of 1997. The concentrations for these samples were between 87 and 89 percent of the expected values. Data packages for the dose conformation samples are located in Task 33 MREF Chemistry Binder 1 and 2.

A Miniature Automatic Continuous Air Monitoring System (MINICAMS®) was utilized for verification of decontamination before the animals were removed from the fume hood. The MINICAMS® is a real-time, on-line data acquisition system that employs a solid-sorbent tube to pre-concentrate the agent vapor (PCT), a capillary gas chromatographic (GC) column for separation, a flame-photometric detector (FPD), and a PC computer for data acqusition. The MINICAMS® is designed primarily for the rapid determination of the 8-hour, time-weighted-average (TWA) concentrations of chemical-warfare (CW) agents and simulants. The verification of decontamination was performed as outlined in the protocol and approved by the MREF Safety Officer.

Experimentally, a plastic bag was securely taped to the skin of the animal covering all of the dosing sites. After the bag had been attached for a minimum of 15 min, an air sample was collected from the bag for 1 min and analyzed using the HDPIG method developed for the MINICAMS® at MREF. At least five different calibration levels were used for the regression analysis. Data packages for verification of decontamination are located in Task 33 MREF Chemistry Binder 3. Each data package includes a copy of the MINICAMS® HDPIG method. calibration results, analysis results for each sample and a chart recording of chromatograms. A

check sample, which is a sample of known HD concentration near 0.5 TWA, was used to verify the instrument's response before and after air samples were acquired.

Initially, when bleach was used to decontaminate the dose sites, several analyses with additional decontamination were required to lower the HD level below 0.5 TWA within the sampling bag. Later, water was used to decontaminate the dose sites and all of the animals passed the decontamination test on the first analysis and no HD was detected in most of the air samples.

All of three Chemistry Study Binders containing raw data have been transferred to Jack Waugh. The data has been QC'd, reviewed and boxed. Archive sheets were completed and included in the box.

M:/ Projects/ Task 94-33/Chemistry/ Reports/Memo September 29, 1999.doc

Clinical Observations and Histological Evaluations of the Wound Site No. 1

This section defines and presents a minimal scoring regimen to use on each lesion for this study. This scoring regimen may need to be adjusted based on observations made during Phase I. Any changes or adjustments will be following discussions and approval by the COR and/or sponsor designee.

I. Definitions

Adherence - the act or quality of sticking to something.

Contraction/Closure - a drawing together, a shortening or shrinkage.

Durability - highly resistant to wear and tear.

Edema - presence of large amounts of fluid in intercellular spaces of the body. Edema encompasses swelling.

Epithelialization - healing by the growth of epithelium over a denuded surface.

Erythema - a name applied to the redness of the skin produced by congestion of the capillaries, which may result from a variety of causes.

Eschar - a slough produced by a thermal burn, corrosive application, or by gangrene.

Exudate - material, such as fluid, cells or cellular debris, which has escaped from blood vessels and is deposited in or on tissues. Exudate are characterized by high protein content, cells, or solid materials derived from cells.

Granulation - the formation in wounds of small, rounded masses of tissue during healing.

Inflammation - a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off both injurious agent and the injured tissue. Inflammation is characterized by pain, heat, redness, and edema.

Necrosis - the sum of morphological changes indicative of cell death and caused by the progressive degradetive action of enzymes.

Rejection - the process of walling off and/or failure to incorporate foreign material. Graft rejection is an immune response against a grafted tissue that results in the failure of the graft to survive. Graft rejection is characterized histologically as an extensive infiltration by mononuclear cells, primarily small lymphocytes, accompanied by edema and interstitial hemorrhage. Observational rejection will be

characterized as nonvascularized material with lack of adherence and accompanied by necrosis.

Sloughing - the formation or separation of necrotic tissue in the process of separating from viable portions of the body. To shed or cast off.

Swelling/Edema - a transient abnormal enlargement or increase in volume of a body part or area not caused by proliferation of cells.

Vascularization - the formation of new blood vessels.

NA - Score NA when observations can not be made and give justification.

II. Observation and Histological Evaluation and Scoring of Each Lesion by Event

The scoring criteria and criteria descriptions may need to be changed, based on observations during Phase I.

A. Wound Site

A-1. Clinical Observation - Initially daily observations are made until adequate observation intervals are identified.

1. Size of Wound - Metric measurement with ruler

2. Exudate

0	None	
1	Minimal -	Less than 1/3 of the wound area is covered.
2	Moderate -	Between 1/3 and 2/3 of the wound area is covered.
3	Maximum -	Greater than 2/3 of the wound area is covered.

The following observations of the wound area should occur after gentle cleaning.

3. Erythema

0	None	
1	Slight -	Light pink to pink, area not well defined.
2	Slight to Moderate -	Pink to light red, well defined area
3	Moderate -	Red, well defined lesion.
4	Moderate to Severe -	Red to deep red (beet red), well defined lesion to spreading = possibly larger than original site.
5	Severe -	Deep red to purple, evidence of necrosis and/or eschar in addition to criteria for 4 above.

4. Edema

0	None	
1	Slight -	Barely perceptible or questionable.
2	Slight to Moderate -	Slightly raised area with well defined edges.
3	Moderate -	Area raised approximately 1 millimeter, well defined.
4	Moderate to Severe -	Area raised greater than 1 millimeter, well defined possibly spreading larger than original site.
5	Severe -	Area raised greater than 1 millimeter and extending beyond area of exposure.

5. General Impression - Overall observer's impressions.

0	None	
1	Slight -	Lesion maybe slightly erythematous or slightly edematous with area not well defined.
2	Slight to Moderate -	Lesion may be erythematous and/or edematous in a well defined area.
3	Moderate -	Lesion may be severely erythematous and/or severely edematous in a well defined or beginning to spread.
4	Moderate to severe -	Lesion may be severely edematous, severely erythematous, and spreading with some evidence of necrosis (exudate).
5	Severe -	Lesion with severe necrosis and/or eschar and may have spread.

A-2. Clinical Observational Wound Severity Score - Function of scores from criteria 2 through 5.

A-3. Histology Evaluation - The wound biopsy is evaluated for full-thickness or deep-partial thickness burn.

B. Test Material or Control Implants and Wounds

B-1. Clinical Wound Healing Observation of Wound Area - Minimally once a week during bandage change.

1. Wound Size - Metric measurement with ruler

2. Exudate - See section II.A.A-1.2.

The following observations of the wound area should occur after gentle cleaning.

3. Granulation

0	None
1	Minimal
2	Moderate
3	Maximum

4. Inflammation

0	None -	No inflammation observed.
1	Slight -	Barely perceptible, light pink to pink, not well defined.
2	Slight to Moderate -	Slightly raised area with well defined edges, pink to light red.
3	Moderate -	Area light red to red and raised approximately 1 millimeter, well defined.
4	Moderate to Severe -	Area red to deep red and raised greater than 1 millimeter, well defined possibly spreading larger than original site.
5	Severe -	Area deep red to purple with evidence of necrosis or eschar and raised greater than 1 millimeter and extending beyond area of exposure.

5. Contraction

0	Maximum
1	Moderate
2	Minimal
3	None

6. Infection

0	Absent
1	Present

B-2. Clinical Observations of Test or Control Articles - Minimally once a week during bandage change.

1. Rejection

0	None -	No rejection
1	Very slight -	Isolated, small areas, less than a quarter of the lesion, indicates material rejection.
2	Slight -	Approximately one quarter to half of the lesion indicates material rejection.
3	Moderate -	Over half of the lesion indicates material rejection.
4	Extensive -	Rejection of material.

2. Adherence

0	No slough -	Adhered, entire TWD is adhered to the lesion.
1	Very slight -	Isolated, small areas less than a quarter of the lesion is sloughing.
2	Slight -	Approximately one quarter to half of the lesion is sloughing.
3	Moderate -	Over half of the lesion is sloughing.
4	Extensive -	Entire lesion sloughed.

3. Durability

0	Extensive durability -	Greater than 2/3 of wound area durable.
1	Moderate durability -	Between 1/3 and 2/3 of wound area durable.
2	Minimal durability -	Less than 1/3 of wound area durable.
3	None -	Total breakdown of TWD

B-3. Clinical Observation Wound Healing Score - Function of scores from B-1., except for Nos. 1., and B-2.

B-4. Histology Evaluation - None.

C. Test or Control Article Removal Day - Removed maximally 2 weeks after implant or earlier if control article is sloughing.

C-1. Clinical Observations of Test or Control Wound Site

1. Wound Size - Metric measurement with ruler

2. Exudate - See section II.A.A-1.2.

The following observations of the wound area should occur after gentle cleaning.

3. Erythema - See section II.A.A-1.3.

Note: This may be deleted if found not relevant or well represented by the criteria section inflammation.

4. Edema - See section II.A.A-1.4.

Note: This may be deleted if found not relevant or well represented by the criteria section inflammation.

5. Granulation - See section II.B.B-1.3.

6. Inflammation - See section II.B.B-1.4.

7. Vascularization

0	Extensive
1	Moderate to Extensive
2	Moderate
3	Slight to Moderate
4	Very Slight
5	None

8. Epithelialization - Rough estimation of percent of closure.

0	Extensive - 100 percent closed.
1	Moderate to Extensive - 75 to 100 percent closed.
2	Moderate - 50 to 75 percent closed.
3	Slight to Moderate - 25 to 50 percent closed.
4	Very Slight - 0 to 25 percent closed.
5	None - 0 percent closed.

9. Contraction - See section II.B.B-1.5.

10. Infection - See section II.B.B-1.6.

11. Degree of Wound Bed Preparation

0	None
1	Minimal
2	Moderate
3	Extensive

C-2. Clinical Observations of Test or Control Article

1. Rejection - See section II.B.B-2.1.

2. Adherence - See section II.B.B-2.2.

3. Durability - See section II.B.B-2.3.

4. Ease of removal of Test or Control Article - Maybe quantitative and scored.

0	Easily Removed - No adherence or sloughed.
1	Slight adherence - Gentle pull removes material.
2	Moderate - Firm pull and some cutting may be required.
3	Difficult - Material must be dissected out.

C-3. Clinical Observation Wound Healing Score - Function of C-1, except Nos. 1, and C-2 scores.

C-4. Histology Evaluation - Of removed test article or control implant and site.

1. Inflammation - Include cell types

0	None - No inflammation observed.
1	Slight
2	Slight to Moderate
3	Moderate
4	Moderate to Severe
5	Severe

2. Vascularization

0	Extensive
1	Moderate to Extensive
2	Moderate
3	Slight to Moderate
4	Very Slight
5	None

3. Epithelialization

0	Extensive
1	Moderate to Extensive
2	Moderate
3	Slight to Moderate
4	Very Slight
5	None

4. Necrosis

0	None
1	Slight
2	Moderate
3	Severe

5. Granulation

0	None
1	Minimal
2	Moderate
3	Maximal

6. Presence of Test or Control Material

0 Absent
1 Present

7. Completeness of Wound Healing Determined by Pathologist

   0 Maximum
   1 Moderate
   2 Minimal
   3 None

D. Autograft

D-1. Clinical Observations of Autograft - Minimally once a week during bandage change.

1. Wound Size - Metric measurement with ruler

2. Exudate - See section II.A.A-1.2.

The following observations of the wound area should occur after gentle cleaning.

3. Rejection - See section II.B.B-2.1.

4. Adherence - See section II.B.B-2.2.

5. Durability - See section II.B.B-2.3.

6. Erythema - See section II.A.A-1.3.

   Note: This may be deleted if found not relevant or well represented by the criteria section inflammation.

7. Edema - See section II.A.A-1.4.

   Note: This may be deleted if found not relevant or well represented by the criteria section inflammation.

8. Granulation - See section II.B.B-1.3.

9. Inflammation - See section II.B.B-1.4.

10. Vascularization - See section II.C.C-1.7.

11. Epithelialization - See section II.C.C-1.8.

12. Necrosis

   0 None
   1 Minimal - Less than 1/3 of the wound area is covered.

2 Moderate - Between 1/3 and 2/3 of the wound area is covered.
3 Maximum - Greater than 2/3 of the wound area is covered.

13. Contraction - See section II.B.B-1.5.

14. Infection - See section II.B.B-1.6.

D-2. Clinical Observation Wound Healing Score - Function of D-1 scores except for .

D-3. Histology Evaluation - Weekly biopsies.

1. Inflammation - See section II.C.C-4.1.

2. Vascularization - See section II.C.C-4.2.

3. Epithelialization - See section II.C.C-4.3.

4. Necrosis - See section II.C.C-4.4.

5. Granulation - See section II.C.C-4.5.

6. Presence of Test or Control Material - See section II.C.C-4.6.

7. Completeness of Wound Healing Determined by Pathologist - See section II.C.C-4.7.

3-28-97 CLINICAL OBSERVATIONS EVALUATION/DEFINITION 2

Clinical Observations and Histological Evaluations of the Wound Site

This section defines and presents a minimal scoring regimen to use on each lesion for this study. This scoring regimen may need to be adjusted based on observations made during Phase I. Any changes or adjustments will be following discussions and approval by the COR and/or sponsor designee.

I. Definitions

Adherence - the act or quality of sticking to something.

Contraction/Closure - a drawing together, a shortening or shrinkage. This is mediated by an interaction of wound myofibroblasts and matrix components. This begins approximately 1 week after injury.

Durability - highly resistant to wear and tear.

Edema - presence of large amounts of fluid in intercellular spaces of the body. Edema encompasses swelling.

Epithelialization - healing by the growth of epithelium over a denuded surface.

Erythema - a name applied to the redness of the skin produced by congestion of the capillaries, which may result from a variety of causes.

Eschar - A scab or a slough produced by a thermal burn, corrosive application, or by gangrene.

Exudate - material, such as fluid, cells or cellular debris, which has escaped from blood vessels and is deposited in or on tissues. Exudate are characterized by high protein content, cells, or solid materials derived from cells.

Granulation - the formation in wounds of small, rounded masses of tissue during healing.

Inflammation - a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute, or wall off both injurious agent and the injured tissue. Inflammation is characterized by pain, heat, redness, and edema.

Necrosis - the sum of morphological changes indicative of cell death and caused by the progressive degradetive action of enzymes.

Rejection - the process of walling off and/or failure to incorporate foreign material. Graft rejection is an immune response against a grafted

tissue that results in the failure of the graft to survive. Graft rejection is characterized histologically as an extensive infiltration by mononuclear cells, primarily small lymphocytes, accompanied by edema and interstitial hemorrhage. Observational rejection will be characterized as nonvascularized material with lack of adherence and accompanied by necrosis.

Sloughing - the formation or separation of necrotic tissue in the process of separating from viable portions of the body. To shed or cast off.

Swelling/Edema - a transient abnormal enlargement or increase in volume of a body part or area not caused by proliferation of cells.

Vascularization - the formation of new blood vessels.

NA - Score NA when observations can not be made and give justification.

II. Clinical Observation and Histological Evaluation and Scoring of Each Lesion by Event

The scoring criteria and criteria descriptions are based on observations during Phase I. This scoring system has been defined so that the lower the score the less severe the wound for wound development and the closer to healing for wound healing.

A. Clinical Observation

A-1. Wound Development - Observations are made weekly until Study day 38 or at the discretion of the Study Director in consultation with the CAR. The following observations of the wound area may occur after gentle cleaning, if necessary.

1. Size of Wound - Metric measurement with ruler

2. Exudate

0 None
1 Minimal - Less than 1/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Moderate - Between 2 and 3/4 of the wound area is covered.
4 Maximum - Greater than 3/4 of the wound area is covered.

3-28-97 CLINICAL OBSERVATIONS EVALUATION/DEFINITION 2

3. Erythema

0 None
1 Minimal - Light pink to light red, area may or may not be well defined.
2 Mild - Reddish, may be well defined lesion.
3 Moderate - Deep red (beet red) with or with some purpleish areas, may be a well defined lesion to spreading = possibly larger than original site.
4 Severe - Red, deep red to purple with whitish areas indicating necrosis and/or eschar in addition to criteria for 3 above.

4. Edema

0 None
1 Minimal - Barely perceptible or questionable.
2 Mild - Slightly raised area may have well defined edges.
3 Moderate - Area raised approximately 1 millimeter, may be well defined or spreading.
4 Severe - Area raised greater than 1 millimeter and extending beyond area of exposure.

5. Necrosis

0 None
1 Minimal - Barely perceptible or questionable. Less than 1/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Moderate - Between 2 and 3/4 of the wound area is covered.
4 Maximum - Greater than 3/4 of the wound area is covered.

6. General Description of the wound sites

7. General Severity of Wound - Add the above scores (2 through 5) for this section.

A-2. <u>After Grafting Test Material or Autograft Implants on Wounds</u> - Weekly observations during bandage change. The following observations of the wound area should occur after gentle cleaning when necessary.

1. Wound Size - Metric measurement with ruler

3-28-97 CLINICAL OBSERVATIONS EVALUATION/DEFINITION 2

2. Exudate -

0 None
1 Minimal - Less than 1/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Moderate - Between 2 and 3/4 of the wound area is covered.
4 Maximum - Greater than 3/4 of the wound area is covered.

3. Erythema

0 None
1 Minimal - Light pink to light red, area may or may not be well defined.
2 Mild - Reddish, may be well defined lesion.
3 Moderate - Deep red (beet red) with or with some purpleish areas, may be a well defined lesion to spreading = possibly larger than original site.
4 Severe - Red, deep red to purple with whitish areas indicating necrosis and/or eschar in addition to criteria for 3 above.

4. Edema

0 None
1 Minimal - Barely perceptible or questionable.
2 Mild - Slightly raised area may have well defined edges.
3 Moderate - Area raised approximately 1 millimeter, may be well defined or spreading.
4 Severe - Area raised greater than 1 millimeter and extending beyond area of exposure.

5. Necrosis

0 None
1 Minimal - Barely perceptible or questionable. Less than 1/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Moderate - Between 2 and 3/4 of the wound area is covered.
4 Maximum - Greater than 3/4 of the wound area is covered.

6. Eschar

0 None
1 Minimal - Barely perceptible or questionable. Less than 1/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Moderate - Between 2 and 3/4 of the wound area is covered.
4 Maximum - Greater than 3/4 of the wound area is covered.

3-28-97 CLINICAL OBSERVATIONS EVALUATION/DEFINITION 2

7. Contraction

0 Maximum - Wound has healed to a scar.
1 Moderate - The wound has contracted by about 75 percent or less.
2 Mild - The wound has contracted by about 50 percent or less.
3 Minimal - The wound has contracted by about 25 percent or less.
4 None - No contraction is observed.

8. Infection

0 Absent
1 Present

9. Granulation

0 None
1 Minimal - Barely perceptible or questionable. Less than 1/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Moderate - Between 2 and 3/4 of the wound area is covered.
4 Maximum - Greater than 3/4 of the wound area is covered.

10. Vascularization

0 Extensive - Greater than 3/4 of the wound area is covered.
1 Moderate - Between 2 and 3/4 of the wound area is covered.
2 Mild - Between 1/4 and 2 of the wound area is covered.
3 Slight - Barely perceptible or questionable. Less than 1/4 of wound is covered.
4 None

11. Epithelialization - Rough estimation of percent of closure.

0 Extensive - 100 percent closed.
1 Moderate - 75 percent closed.
2 Mild - 50 percent closed.
3 Slight - 25 percent closed.
4 None - 0 percent closed.

3-28-97 CLINICAL OBSERVATIONS EVALUATION/DEFINITION 2

Graft Sites Only

12. Rejection

0 None - No rejection
1 Very slight - Isolated, small areas, less than a 1/4 of the lesion, indicates material rejection.
2 Slight - Approximately 1/4 to 2 of the lesion indicates material rejection.
3 Moderate - Over 2 to 3/4 of the lesion indicates material rejection.
4 Extensive - Rejection of material.

13. Adherence

0 No slough - Adhered, entire TWD is adhered to the lesion.
1 Very slight - Isolated, small areas less than 1/4 of the lesion is sloughing.
2 Slight - Approximately 1/4 to 2 of the lesion is sloughing.
3 Moderate - Over 2 to about 3/4 of the lesion is sloughing.
4 Extensive - Entire lesion sloughed.

14. Durability

0 Extensive durability - TWD is durable, resistant to wear or decay.
1 Moderate durability - Isolated, small areas, less than a 1/4 of the lesion is non-durable.
2 Mild durability - Approximately 1/4 to 2 of the lesion is non-durable.
3 Minimal durability - Over 2 to 3/4 of the lesion is non-durable.
4 None - Total breakdown of TWD.

15. Wound Healing Score - Function of scores from A-2., except for Nos. 1, 11., 12. and 13. Graft scores can be added separately and compared between each type of graft.

C. Histology Evaluation

C-1. Histology of Wound Development Site - Determined in Phase I on Day 2 prior to grafting in the last 6 animals dosed.

C-2. Histology Evaluation of Wound Healing - To be determined by Pathologist.

Clinical Observation Evaluation/Definition 3 Worksheet
Used on Phase III

1. Size of wound

    a. Length (mm)
    b. Breadth (mm)

    - calipers will be used to make the measurements
    - length = anterior (cranial) to posterior (caudal); 9 to 3 o'clock
    - breadth = left to right; 12 to 6 o'clock - measured over the area of erythema or scab, but not the area of edema
    - on surgical wounds, the whole wound is measured, not just the HD-dosed area; as the edge of the wound heals (e.g., day 14-21), measure just the remaining scab-covered area (e.g., the periphery of a dermatomed area blends into normal skin over time, and cannot be easily measured)

2. Exudate

    0 = absent
    1 = present but moist
    2 = present as dried scab (e.g., crusty, especially around edges)
    * = can't evaluate, due to eschar or other condition

3. Eschar (slough made of several cell layers, usually visible by day 7)

    0 = absent
    1 = present

4. Percent Area Covered by Eschar or Scab

    0 = none
    1 = less than 25% of original dosing area involved
    2 = at least 25% but less than 50% of original dosing area involved
    3 = at least 50% but less than 75% of original dosing area involved
    4 = 75% or greater of original dosing area involved

5. Extent of Erythema (pink, red or deep red)

0 = none present
1 = present along border or along border and within border
2 = beyond border, and inclusive of #1
* = not observable, due to scab, eschar or other condition

Note: The comment section can be used for more in-depth descriptions.

6. Description of Erythema (darkest hue present)

0 = none
1 = pink
2 = red
3 = deep red

7. Hemorrhage (purple)

0 = absent
1 = present
* = not observable, due to scab, eschar or other condition

8. Edema (measurements made via calipers)

a. Height (mm above teat line?)
b. Length (mm)
c. Breadth (mm)
d. Visual Score:

0 = none
1 = minimal - barely perceptible or questionable
2 = mild - area raised approximately 1 mm, may have well defined edges
3 = moderate- area raised approximately 2 to 3 mm, well defined and may be spreading
4 = severe - area raised 4 mm or more, and extending beyond area of exposure

9. Extent of Necrosis (white patches)

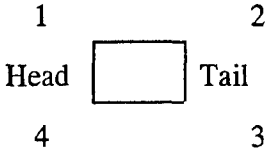
0 = none
1 = less than 25% of original dosing area involved
2 = at least 25% but less than 50% of original dosing area involved
3 = at least 50% but less than 75% of original dosing area involved
4 = 75% or greater of original dosing area involved
* = not 0, but cannot be adequately observed due to coverage by scab, graft, or other condition

10. Infection (suppuration)

0 = absent
1 = present

11. Wound contraction

a. Place a tattoo mark (small "+" or "X") beside each of the two anterior-most dosing sites, and beside each of the two posterior-most dosing sites. The tattoos should be at least 1.5 cm beyond the edge of each of those four 5 x 5 cm delineated sites, and positioned midway between the dorsal and ventral outer corners of the sites. The tattoo marks should be mentally numbered as follows:

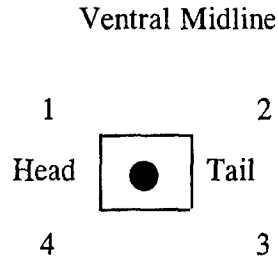


b. The following measurements will be made with a metric ruler (mm) between the centers of the tattoo marks:

1 → 2
2 → 3
3 → 4
4 → 1
1 → 3
2 → 4

These measurements will be used, along with body weight measurements, to judge the overall growth of the animal during the course of the experiment.

c. On day of surgical manipulation (e.g., day 2), place tattoo marks (small +'s or X's) just to the outside of all four corners of each lesion or surgical graft site, in a consistent manner. The tattoo spots are to be mentally numbered as follows:



d. The following measurements will be made with a caliper (mm) between the centers of the tattoo marks on each site:

1 → 3
2 → 4
1 → edge of lesion, along the diagonal
2 → edge of lesion, along the diagonal
3 → edge of lesion, along the diagonal
4 → edge of lesion, along the diagonal

These measurements will be used to ascertain if the wounds have contracted over time.

12. General health

a. weight (kg)
b. rectal and room temperatures (take immediately after induction of anesthesia)
c. respiratory/ENT problems (e.g., sneezing, runny nose)
d. gastrointestinal problems (e.g., diarrhea)
e. skin problems (not related to exposure sites)
f. other

13. Comments

ATTACHMENT I

Summary Tables of Animal Health and Animals Used on Each Phase

Table I-1. Task 94-33 Swine Deaths Summary

Animal Number	Summary of Death	Animal Replaced
97-5-9	Died 3-14-97, GI bloat, rupture of gut, and partial torsion of colon	Yes
97-11-11	Died 3-26-97, Respiratory distress – complications of anesthesia and swollen larynx	Yes
97-6-8	Died 4-3-97, GI bloat, small intestinge protruded through tear in abdominal wall adjacent to wound scar (site F) and rectal prolapse (repaired)	Yes
97-9-5	Died 4-19-97, Aspiration of feed or porcine stress syndrome	Yes
97-34-5	Euthanized 5-23-97 during quarantine, pulmonary pneumonia – received with respiratory disease	No*
97-43-12	Died 6-24-97, GI bloat, small intestine protrudes through tear adjacent to rectum and rectal prolapse	Yes
97-46-14	Died 6-27-97, GI bloat and volvulus	Yes

*Animal not placed on study

Table I-2. Task 94-33 Swine Health Problems

Animal Number	Health Problems
97-48-11	Highly excitable upon receipt (6-30-97). Prolapsed
97-47-6	Receipt fine (6-30-97), Prolapse
97-51-8	Receipt (7-7-97). Upper respiratory audible chest sounds
97-6-8	Died GI bloat and rectal prolapse
97-11-11	Died GI bloat and rectal prolapse

Table I-3. Task94-33: Animal Identification Listing With Dose Date

Phase I		Phase II	
Animal Nos.	Dose Date	Animal Nos.	Dose Date
96-13-9	4-10-96	97-6-8	3-10-97
96-13-7	4-24-96	97-5-9	3-11-97
96-21-7	5-6-96	97-9-5	3-17-97
96-21-10	5-14-96	97-11-11	3-24-97
96-34-5	6-11-96	97-9-6	3-25-97
96-34-4	6-25-96	97-104-10	3-26-97
96-1-4	8-19-96	97-11-7	3-31-97
96-1-3	8-21-96	97-11-9	4-2-97
96-3-9	8-26-96	97-12-9	4-7-97
96-2-10	8-28-96	97-12-2	4-8-97
96-5-8	9-4-96	97-17-6	4-21-97
96-10-12	9-16-96	97-18-5	4-22-97
96-10-11	9-17-96	97-22-5	5-5-97
96-11-8	9-23-96	97-21-5	5-6-97
96-11-11	9-24-96	97-29-7	5-12-97
96-18-8	10-15-96	97-29-5	5-13-97
		97-31-11	5-19-97
96-24-8	11-11-96	97-31-13	5-20-97
96-22-3	11-13-96	97-34-5	NA*
96-28-12	11-18-96	97-31-12	5-27-97
96-28-13	11-19-96	97-34-7	6-3-97
96-30-5	12-2-96	97-43-12	6-16-97
96-31-10	12-3-96	97-46-13	6-24-97
		97-44-11	6-17-97
97-50-14	2-3-97	97-46-14	6-23-97
97-50-16	2-4-97	97-47-6	7-7-97
97-60-11	2-10-97	97-48-11	7-8-97
97-63-10	2-11-97	97-51-5	7-14-97
97-1-7	2-17-97	97-51-8	7-15-97
97-1-4	2-18-97	97-54-11	7-21-97
		97-103-11	7-22-97

* Animal 97-34-5 was euthanatized during quarantine for a respiratory infection upon receipt

NA means not applicable

* animal not placed on study

TableI-4. Task 94-33: Animal Identification Listing with Dose Date and SM Exposure Time

PHASE III			
PART B			
Animal Number	Start Date		
99-2-10	2-15-99		
99-2-11	2-16-99		
99-23-12	2-10-99		
99-2-9	2-15-99		
99-55-6	2-10-99		
99-6-1	2-16-99		
PART C			
Animal Number	Dose Date	Animal Number	Dose Date
Pilot Animals			
99-299-9	5-10-99	99-118-4	5-11-99
99-119-5	5-17-99	99-30-13	5-18-99
99-45-12	6-7-99	99-45-3	6-8-99

Study Animals					
Animal Nos.	SM Exposure Time (min)	Dose Date	Animal Nos.	SM Exposure Time (min)	Dose Date
99-57-10	30	6-14-99	99-56-7	30	6-15-99
99-55-9	0	6-21-99	99-225-1	0	6-22-99
99-60-2	2	6-28-99			
99-60-4	0	7-6-99	99-158-6	0	7-7-99
99-161-8	30	7-19-99	99-66-11	30	7-20-99
99-73-9	2	7-26-99	99-70-10	2	7-27-99
99-168-8	30	8-2-99	99-75-5	2	8-3-99
99-2-8	2	8-9-99	99-172-10	0	8-10-99
99-203-6	0	8-16-99	99-8-8	0	8-17-99
99-205-5	30	8-23-99	99-205-2	2	8-24-99

ATTACHMENT J

Histopathology Results and Tables for Phase III Part C Pilot Study

TABLE J-1. Phase III Part C Pilot Animals Exposure Times Per Site

Sites		99-299-9 Dose Times (Min)		99-118-4 Dose Times (Min)		99-119-5 Dose Times (Min)		99-30-13 Dose Times (Min)		99-45-3 Dose Times (Min)		99-45-12 Dose Times (min)	
A	B	120	20	10	25	60	40	5	20	30	3	5	1
C	D	80	60	30	5	100	120	15	10	4	1	30 s	3
E	F	40	100	20	15	20	80	25	30	2	5	4	2

S = SECONDS

Table J-2 Histopathology Evaluation Data for Phase III Part C Pilot Animals

Animal Number	Site ID	Time Of Site Exposure	Depth of Necrosis[a]	Necrosis Of Basal Cells[b]	Ulceration	Granulation Tissue	Re-epithelialization
99-30-13	A	5 min	3	4	0	0	0
	B	20 min	4	4	0	0	0
	C	15 min	3	4	0	0	0
	D	10 min	3	4	0	0	0
	E	25 min	4	4	0	0	0
	F	30 min	4	4	0	0	0
	C1	0	0	0	0	0	0
	C2	0	0	0	0	0	0
99-119-5	A	60 min	4	4	0	0	0
	B	40 min	3	4	0	0	0
	C	100 min	4	4	0	0	0
	D	120 min	4	4	0	0	0
	E	20 min	1	4	0	0	0
	F	80 min	4	4	0	0	0
	C1	0	0	0	0	0	0
	C2	0	0	0	0	0	0
99-118-4	A	10 min	3	4	1	0	0
	B	25 min	2	4	0	0	0
	C	30 min	4	4	0	0	0
	D	5 min	2	4	0	0	0
	E	20 min	4	4	0	0	0
	F	15 min	2	4	0	0	0
	C1	0	0	0	0	0	0
	C2	0	0	0	0	0	0
99-299-9	A	120 min	4	4	0	0	0
	B	20 min	4	4	0	0	0
	C	80 min	4	3	0	0	0
	D	60 min	4	4	0	0	0
	E	40 min	4	4	0	0	0
	F	100 min	4	3	0	0	0
	C1	0	0	0	0	0	0
	C2	0	0	0	0	0	0
99-45-12	A	5 min	3	4	0	0	-
	B	1 min	2	1	0	0	-
	C	30 sec	1	1	0	0	-
	D	3 min	3	3	1	0	0
	E	4 min	3	4	0	0	-
	F	2 min	2	2	0	0	-
	C1	0	0	0	0	0	-
	C2	0	0	0	0	0	-

Table J-2 Histopathology Evaluation Data for Phase III Part C Pilot Animals (Continued)

Animal Number	Site ID	Time Of Site Exposure	Depth of Necrosis[a]	Necrosis Of Basal Cells[b]	Ulceration	Granulation Tissue	Re-epithelialization
99-45-3	A	30 sec	1	2	1	0	0
	B	3 min	2	3	0	0	-
	C	4 min	2	3	1	0	0
	D	1 min	1	1	0	0	-
	E	2 min	2	2	0	0	-
	F	5 min	3	4	0	0	-
	C1	0	0	0	0	0	-
	C2	0	0	0	0	0	-

[a]Depth of necrosis scoring: 0 = None, 1 = Squamus epithelium only, 2 = Follicular structures involved, 3 = Not into subcutis, but most of dermis, and 4 = Into subcutis (under depth of necrosis).

[b]Necrosis of Basal Cells scoring: 0 = None, - = None visible, but granulation tissue present, indicating a previous necrotic injury had occurred., 1 = < 5% of area involved, 2 = 10-40% of area involved, 3 = 50-80% of area involved, and 4 = >90% of area involved.

[c]Ulceration: 0 = Absent and 1 = Present

[d]Granulation: 0 – None, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Severe

[e]R0e-epithelialization: 0 = None (epithelial defect present with no re-epithelialization), - = Epithelium not lost yet, 1 = <5% of area from wound margin to cut end of section covered, 2 = 10-40% of area from wound margin to cut end of section covered, 3 = 50-80% of area from wound margin to cut end of section covered, and 4 = >90% of area from wound margin to cut end of section covered.

Table J-3. Depth of Burn Data for Phase III Part C Pilot Animals

Animal Number	Site ID	Time Of Site Exposure	Depth of Burn mm
99-30-13	A	5 min	0.47
	B	20 min	0.55
	C	15 min	0.48
	D	10 min	0.40
	E	25 min	0.70
	F	30 min	0.80
	C1	0	0
	C2	0	0
99-119-5	A	60 min	1.00
	B	40 min	0.60
	C	100 min	*
	D	120 min	0.40
	E	20 min	0.35
	F	80 min	0.50
	C1	0	0
	C2	0	0
99-118-4	A	10 min	0.20
	B	25 min	0.50
	C	30 min	0.50
	D	5 min	0.05
	E	20 min	0.30
	F	15 min	0.25
	C1	0	0
	C2	0	0
99-299-9	A	120 min	0.50
	B	20 min	0.45
	C	80 min	*
	D	60 min	0.30
	E	40 min	0.60
	F	100 min	*
	C1	0	0
	C2	0	0

Table J-3. Depth of Burn Data for Phase III Part C Pilot Animals
(Continued)

Animal Number	Site ID	Time Of Site Exposure	Depth of Burn mm
99-45-12	**A**	**5 min**	0.30
	B	**1 min**	0.01
	C	**30 sec**	0
	D	**3 min**	0.20
	E	**4 min**	0.30
	F	**2 min**	0.15
	C1	**0**	0
	C2	**0**	0.05
99-45-3	**A**	**30 sec**	*
	B	**3 min**	0.50
	C	**4 min**	*
	D	**1 min**	0
	E	**2 min**	0.10
	F	**5 min**	*
	C1	**0**	*
	C2	**0**	0

* = No slide for reading

ATTACHMENT K

Histopathology Report for Lung and Kidney Results of Phase III, Part C, Provided by the United States Army Medical Research Institute of Chemical Defense

VETERINARY PATHOLOGY REPORT

U.S. Army Medical Research Institute
of Chemical Defense
Aberdeen Proving Grounds, MD 21010-5425

ACCESSION NUMBER:
99-0909 thru 99-0918

Pathologist:	Investigator:	Protocol number:	Animal ID:
Dr. Mitcheltree	Dr. Reid	Task 33	unknown

Species:	Breed/Strain:	Sex:	Age:	Date of Death:	Date of Necropsy:
Pig	Yorkshire	Fe	weanling		

HISTORY:
Tissues submitted from Battelle.

GROSS FINDINGS:
None provided.

MICROSCOPIC DIAGNOSIS(ES):
#99-0909: Lung: Hemorrhage, intraalveolar, multifocal, mild. Kidneys: Essentially normal tissues.

#99-0910: Lung: Edema, intraalveolar, multifocal, mild. Kidneys: Essentially normal tissue.

#99-0912: Lung: Edema and congestion, multifocal, moderate, with multifocal intraalveolar histiocytic aggregates; interlobular edema, diffuse, moderate. Kidneys: Essentially normal tissue.

#99-0913: Lung: Moderate, subpleural and interlobular edema; congestion, diffuse, moderate; intraalveolar edema, multifocal, moderate. Kidneys: Essentially normal tissue.

#99-0914: Lung: Congestion and edema, interlobular and intraalveolar, moderate, diffuse, with intrabronchiolar edema and hemorrhage. Kidneys: Essentially normal tissue.

#99-0915: Lung: Congestion and edema, interlobular and intraalveolar, moderate, diffuse, with intrabronchiolar edema and hemorrhage. Kidneys: Essentially normal tissue.

#99-0916: Lung: Congestion and edema, interlobular and intraalveolar, moderate, diffuse, with intrabronchiolar edema and hemorrhage. Kidneys: Essentially normal tissue.

#99-0917: Lung: Congestion and edema, interlobular and intraalveolar, moderate, diffuse, with intrabronchiolar edema and hemorrhage. Kidneys: Essentially normal tissue.

#99-0918: Lung: Congestion and edema, interlobular and intraalveolar, moderate, diffuse, with intrabronchiolar edema and hemorrhage. Kidneys: Essentially normal tissue.

COMMENTS:
Lung changes are probably a result of hypostatic congestion. There was no evidence of necrosis or

VETERINARY PATHOLOGY REPORT

U.S. Army Medical Research Institute
of Chemical Defense
Aberdeen Proving Grounds, MD 21010-5425

ACCESSION NUMBER:
99-0909 thru 99-0918

(continued)
inflammatory cell infiltrate (neutrophils). The presence of blood in the airways is most likely prosector-induced.

REPORTED BY:

Larry W. Mitcheltree, V.M.D.
Veterinary Pathologist
Diplomate, ABT
Comparative Pathology Branch

DATE: 12/27/99

ANIMAL NECROPSY RECORD

ACCESSION NO. 99-0909 to 99-0918

Pathologist Doxtperman Mitchelltree	Investigator Reid	Protocol# Task 33	Animal I.D. #
Species Swine	Strain	Sex / Age	Weight (Gms)
History:		Date Submitted to Lab: 3 Sep 99	
		Date of Necropsy:	
		Scheduled	Found
		Spontaneous Death	Autolyzed
		Other:	Already Necropsied X
Prosector:	Trimmed by: MPS 14 Sept	Photographs	Serology
		Bacteriology	Parasitology

Tissues:	Taken	Trimmed	Cassette	Number
Brain				
Pituitary Gland				
Spinal Cord				
Peripheral Nerve(s)				
Harderian/Lacrimal				
Eye				
Trachea				
Esophagus				
Thyroid & para				
Salivary Gland				
Mandibular LN				
Lungs 205		✓	1	1
Heart & Aorta				
Thymus				
Diaphragm				
Spleen				
Adrenals				
Kidneys (L)		✓	2	1
Liver				
Gallbladder				
Stomach				
Mesenteric LN				
Duodenum				
Jejunum				
Ileum				
Pancreas				
Cecum				
Colon				
Urinary Bladder				
Testicles				
Prostate/Epididymis				
Ovaries/Uterus				
Thigh Muscle				
Sternum or Femur				
Tongue				
Turbinates				
Skin				
Kidney (R)		✓	3	1

99-0909 = 99-203-6
99-0910 = 99-205-5
99-0911 = 99-70-10
99-0912 = 99-205-2
99-0913 = 99-75-5
99-0914 = 99-168-8
99-0915 = 99-172-10
99-0916 = 99-2-8
99-0917 = 99-8-3
99-0918 = 99-75-9

Date Tissue Submitted to Histo Lab or Contractor: 16 Sept 99

Date Slides Submitted to Pathologist for Review: 22 Oct 99

VETERINARY PATHOLOGY REPORT

U.S. Army Medical Research Institute
of Chemical Defense
Aberdeen Proving Grounds, MD 21010-5425

ACCESSION NUMBER:
99-0709 thru 99-0715

Vendor:	Date received:	Investigator:	Protocol number:
		Dr. Reid	Task 33

Species:	Breed/Strain:	Sex:	Age:	Animal ID:
Pig		?	?	Assorted

Date of Death:	Date of Necropsy:	Prosector:
		M. Saulynas

HISTORY:

Animals were part of a study being supported by Battelle (Task 33) and involved cutaneous application of HD.

GROSS FINDINGS:

Sections of kidney and lung were submitted for routine examination. Gross necropsy was performed at Battelle. No gross changes were mentioned.

MICROSCOPIC DIAGNOSIS(ES):

#99-0709 (99-57-10):
Kidneys: Essentially normal tissues.
Lung: Fresh blood in some bronchioles (prosector induced?)

#99-0710 (99-56-7):
Kidneys: Essentially normal tissues.
Lung: Moderate congestion and interlobular edema; moderate, focally-extensive intraalveolar edema (fibrin) with macrophages.

#99-0711 (99-60-2):
Kidneys: Essentially normal tissue.
Lung: Moderate, interlobular edema; focally-extensive congestion; mild, multifocal intraalveolar edema (fibrin); blood in bronchioles (prosector induced?).

#99-0712 (99-60-4):
Kidneys: Essentially normal tissue.
Lung: Moderate, diffuse congestion; mild, multifocal intraalveolar edema (fibrin).

#99-0713 (99-55-9):
Kidneys: Essentially normal tissue.
Lung: Moderate, diffuse congestion; mild, multifocal intraalveolar edema (macrophages, fibrin); moderate interlobular edema.

#99-0714 (99-158-6):

VETERINARY PATHOLOGY REPORT

U.S. Army Medical Research Institute
of Chemical Defense
Aberdeen Proving Grounds, MD 21010-5425

ACCESSION NUMBER:
99-0709 thru 99-0715

(continued)

Kidneys: Essentially normal tissue.
Lung: Moderate, diffuse congestion; moderate interlobular edema; multifocal, moderate intraalveolar edema.

- #99-0715 (99-225-1):
Kidneys: Essentially normal tissue.
Lung: Focally extensive congestion; multifocal, moderate intraalveolar edema (fibrin, macrophages); moderate interlobular edema.

COMMENTS:

Lung changes are probably a result of hypostatic congestion. There was no evidence of necrosis or inflammatory cell infiltrate (neutrophils). The presence of blood in the airways is most likely prosector-induced.

REPORTED BY:

Larry W. Mitcheltree, V.M.D.
Veterinary Pathologist
Diplomate, ABT
Comparative Pathology Branch

DATE: 11/10/99

Figure L-1: Day 0, A, C, E Sites for 2 min Sulfur-Mustard Exposure in Animal 99-60-2

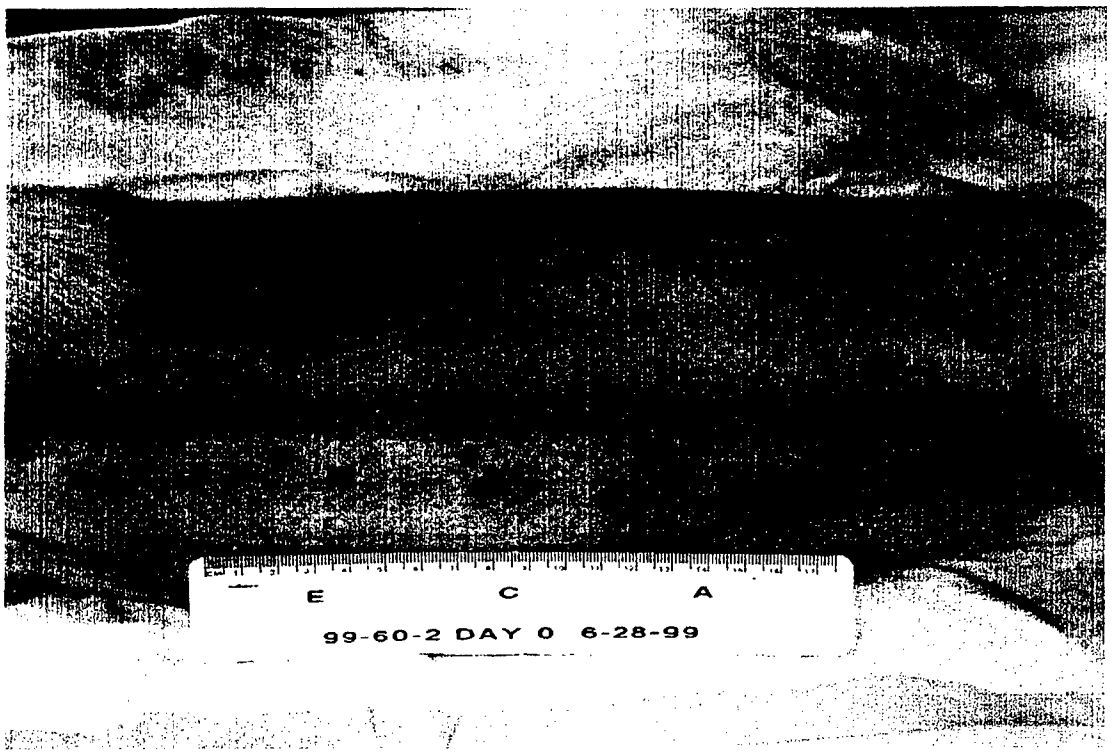


Figure L-2: Day 2 A, C, E Sites for 2 min Sulfur-Mustard Exposure in Animal 99-60-2

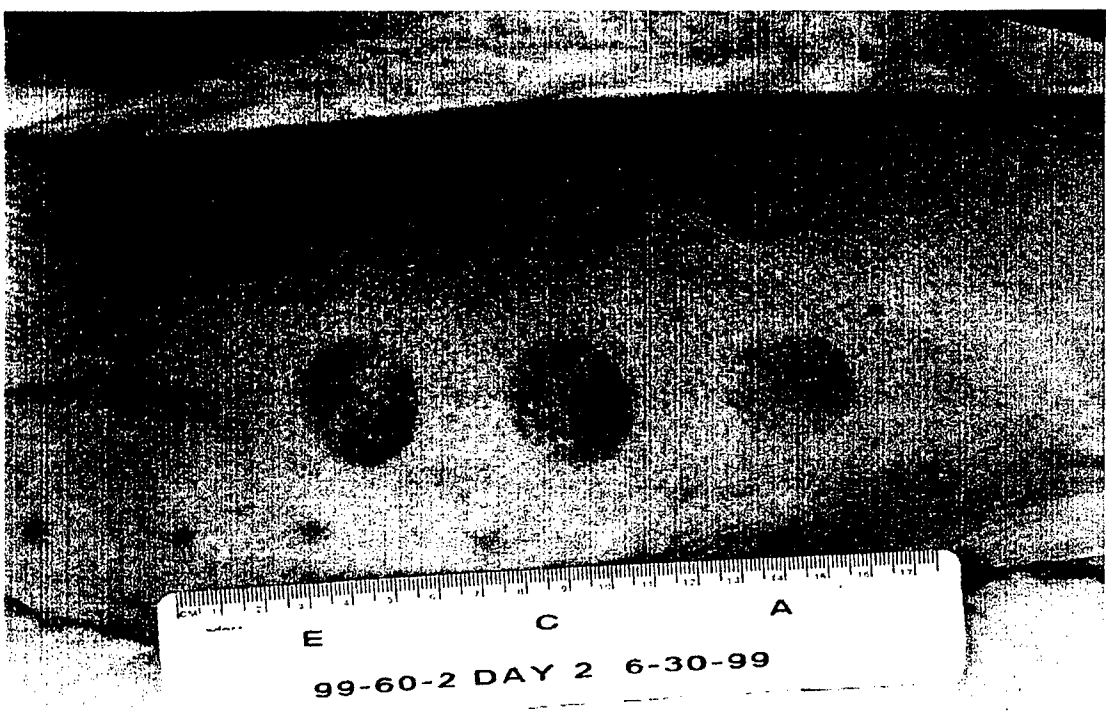


Figure L-3: Day 0 B, D, F Sites for 2 min Sulfur-Mustard Exposure in Animal 99-60-2

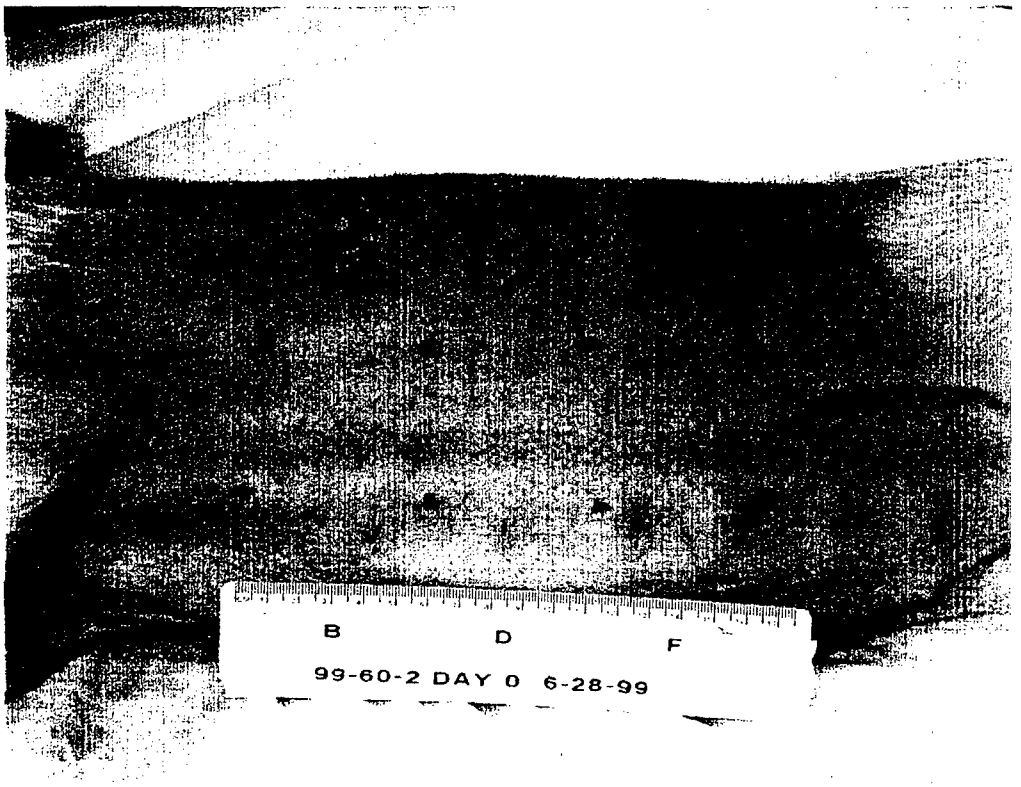


Figure L-4: Day 2 B, D, F Sites for 2 min Sulfur-Mustard Exposure in Animal 99-60-2

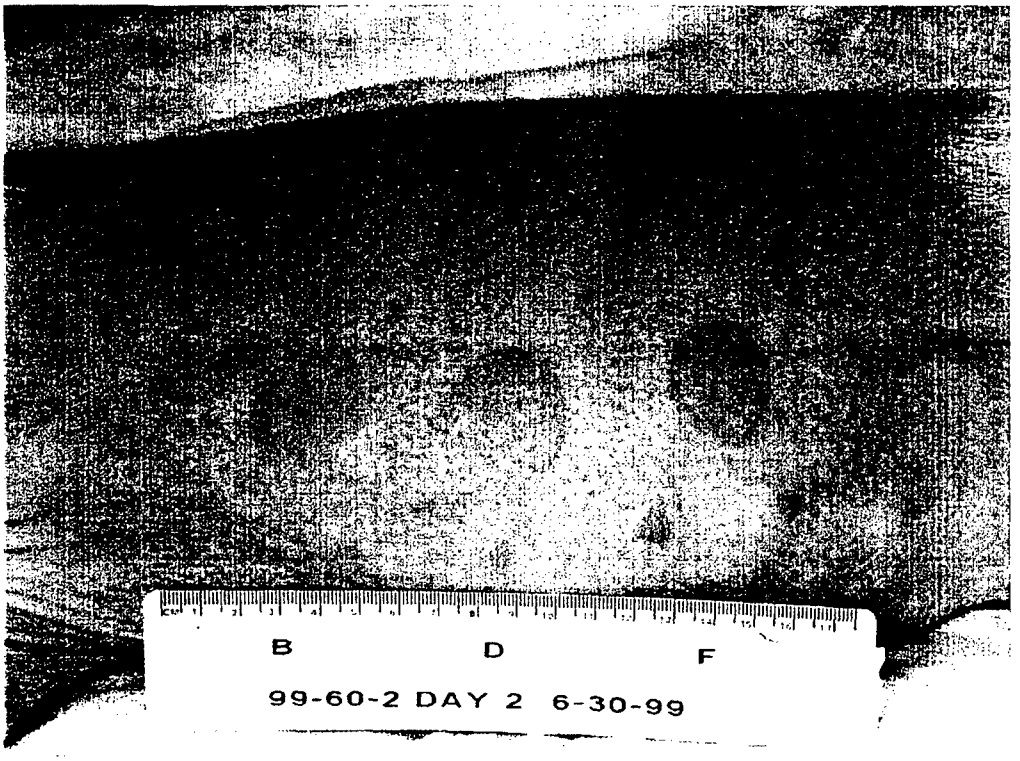
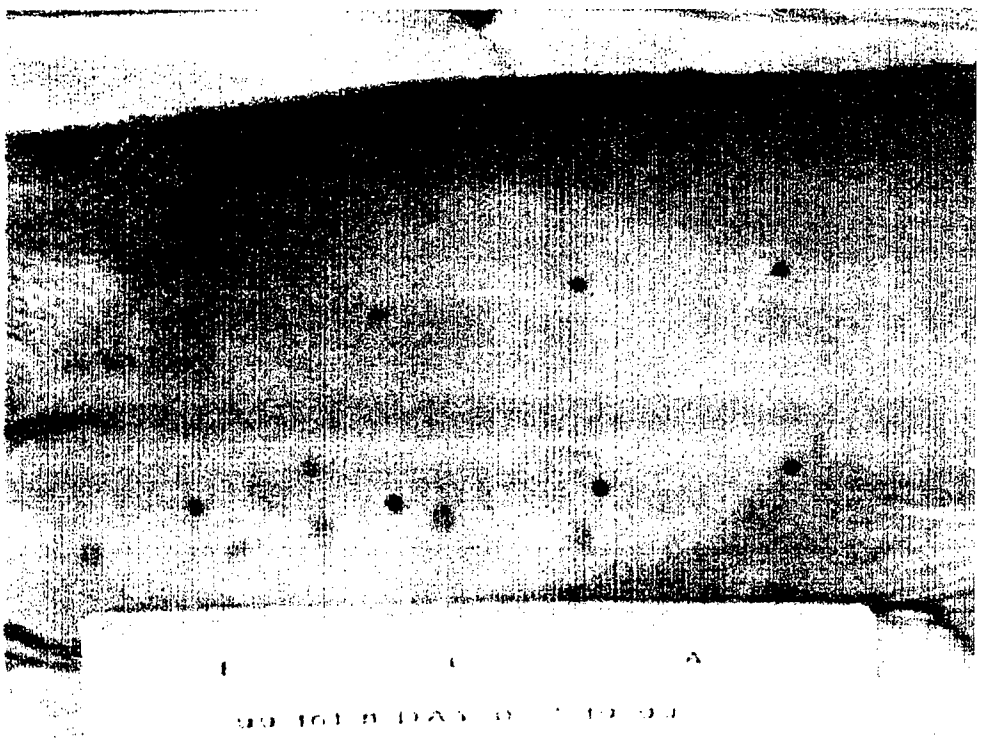


Figure L-5: Day 0 A, C, E Sites for 30 min Sulfur Mustard Exposure in Animal 99-161-8



igure L-6: Day 2 A, C, E Sites for 30 min Sulfur Mustard Exposure in Animal 99-161-8

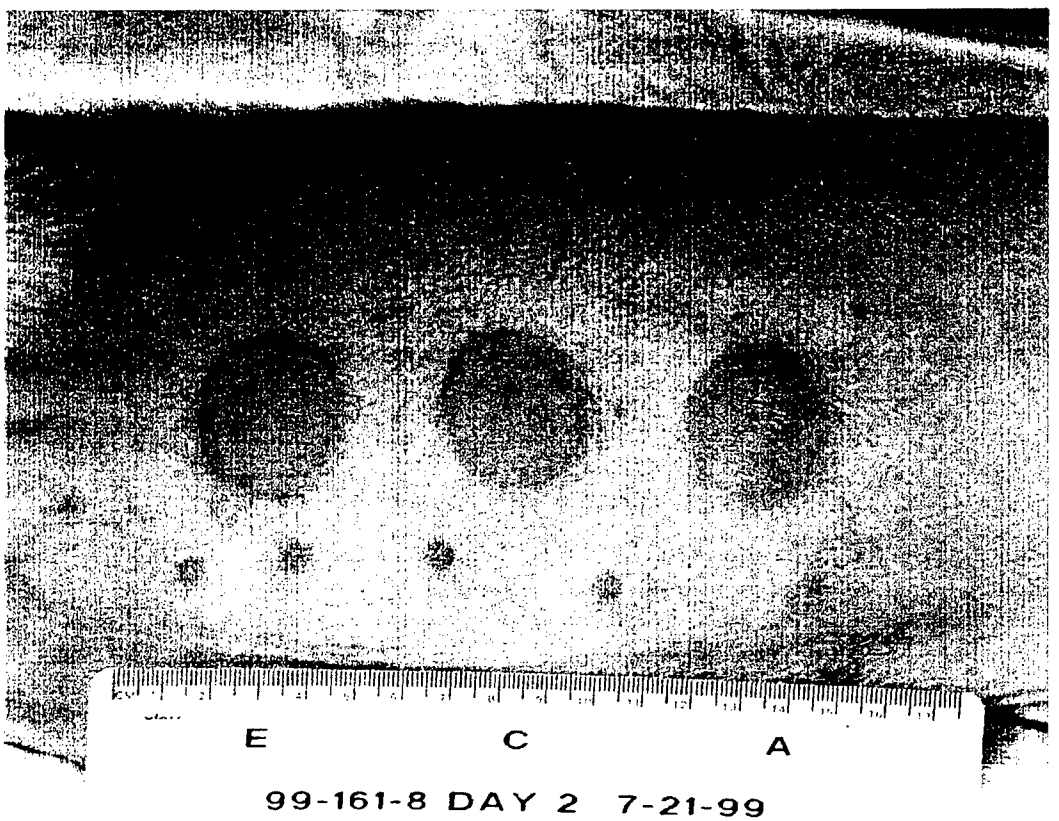


Figure L-7: Day 0 B, D, F Sites for 30 min Sulfur-Mustard Exposure in Animal 99-161-8

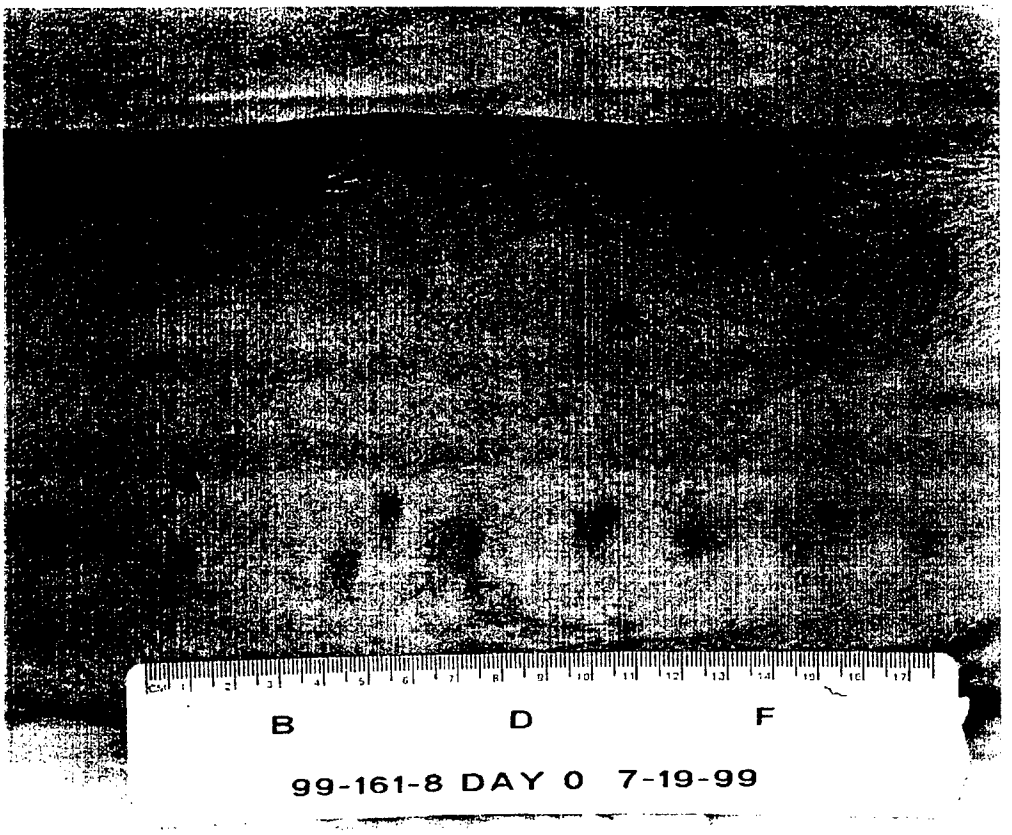


Figure L-8: Day 2 B, D, F Sites for 30 min Sulfur-Mustard Exposure in Animal 99-161-8

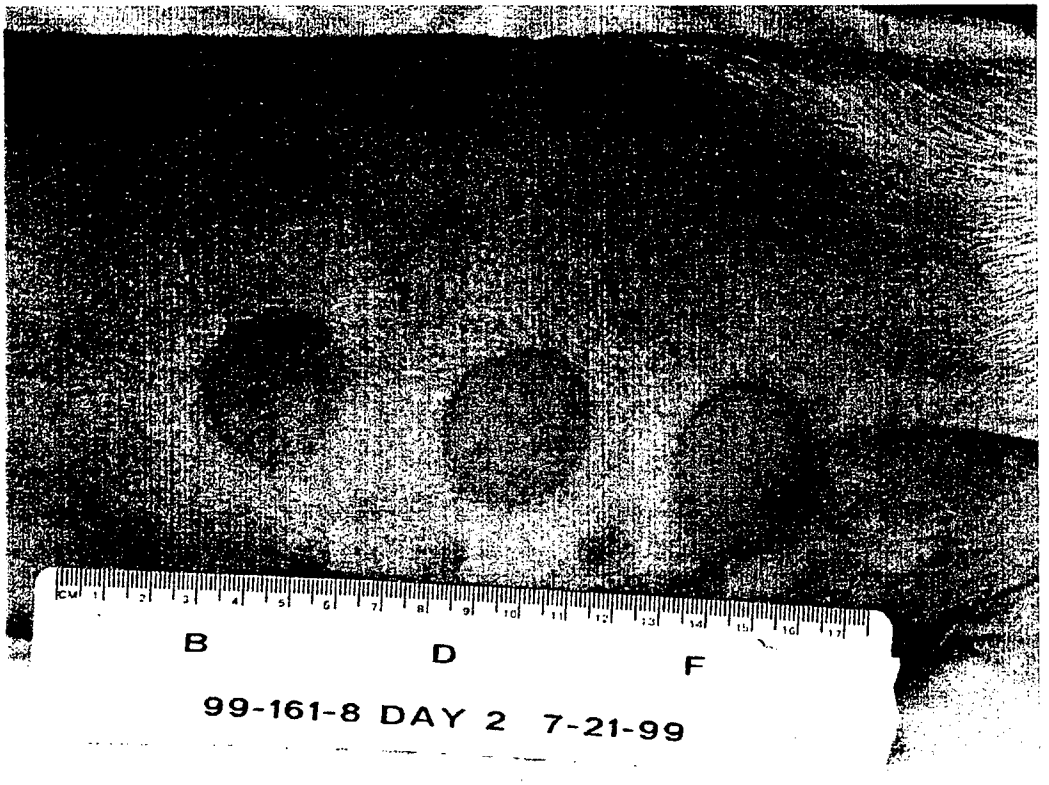


Figure L-9: Day 0 A, C, E Sites for Millipore Water Control Exposure in Animal 99-203-6

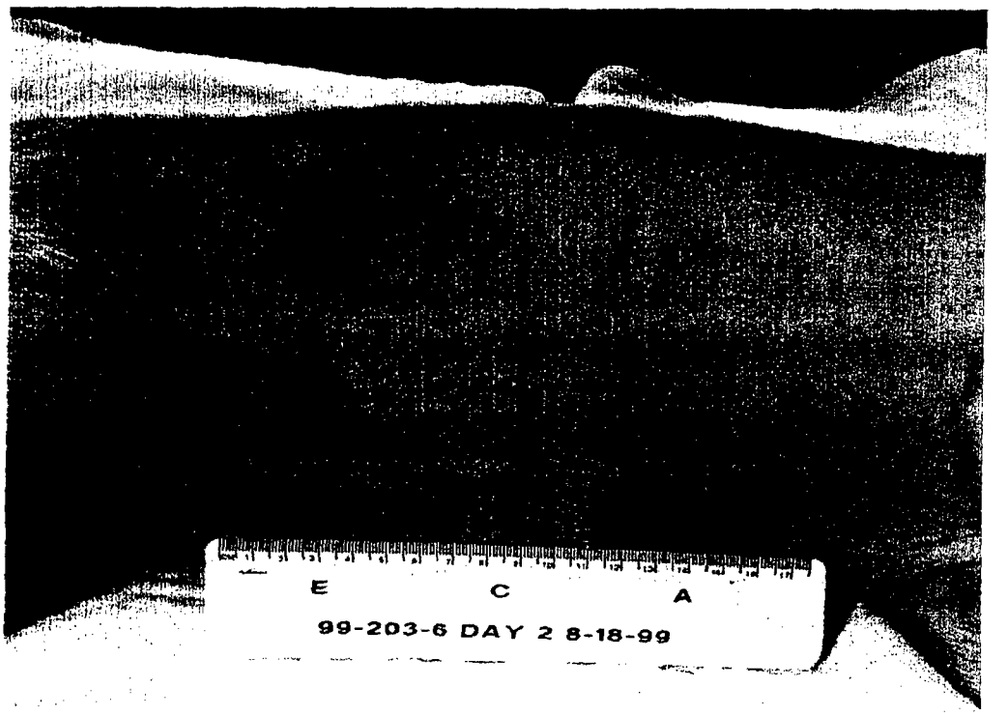


Figure L-10: Day 2 A, C, E Sites for Millipore Water Control Exposure in Animal 99-203-6

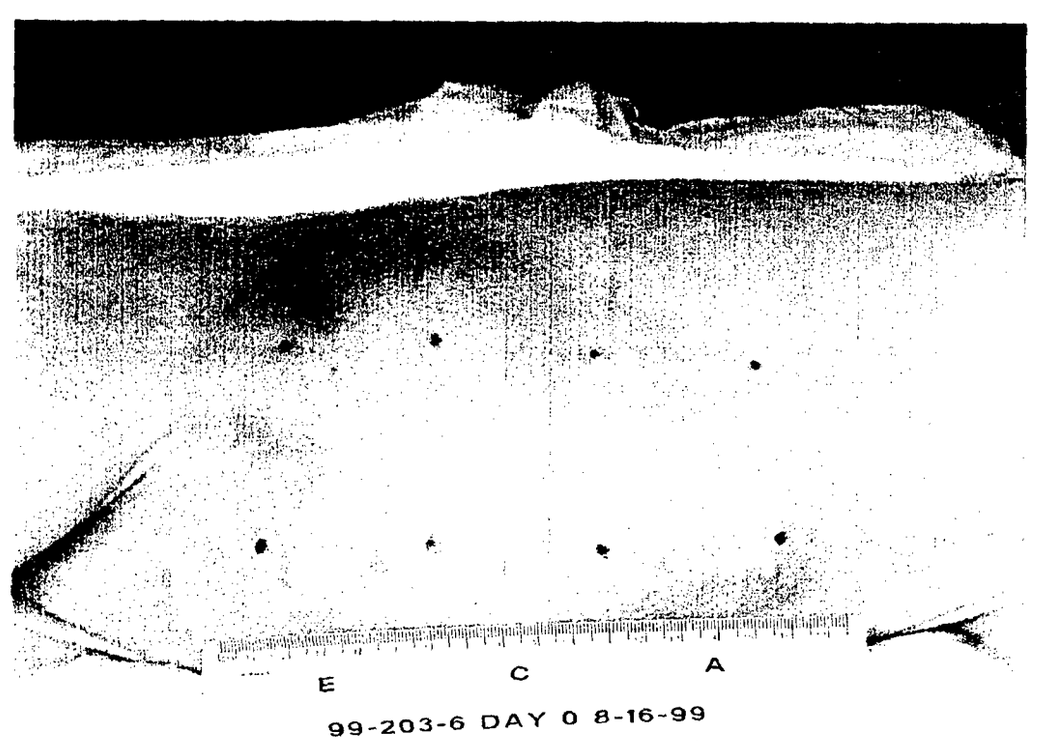


Figure L-11: Day 0 B, D, F Sites for Millipore Water Control Exposure in Animal 99-203-6

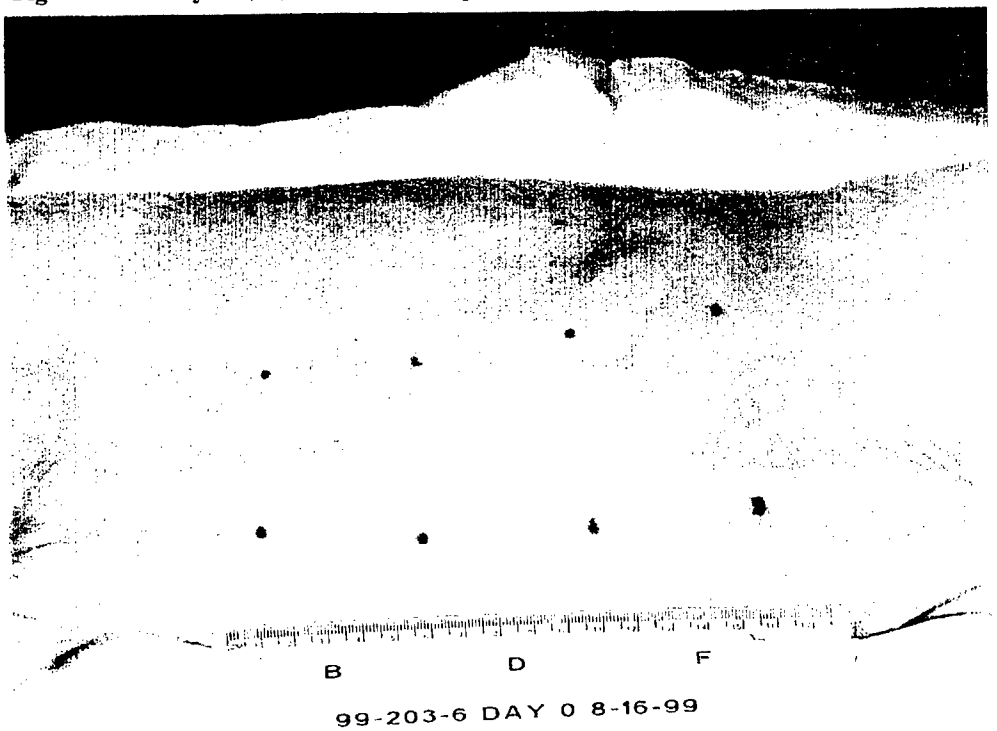


Figure L-12: Day 2 B, D, F Sites for Millipore Water Control Exposure in Animal 99-203-6

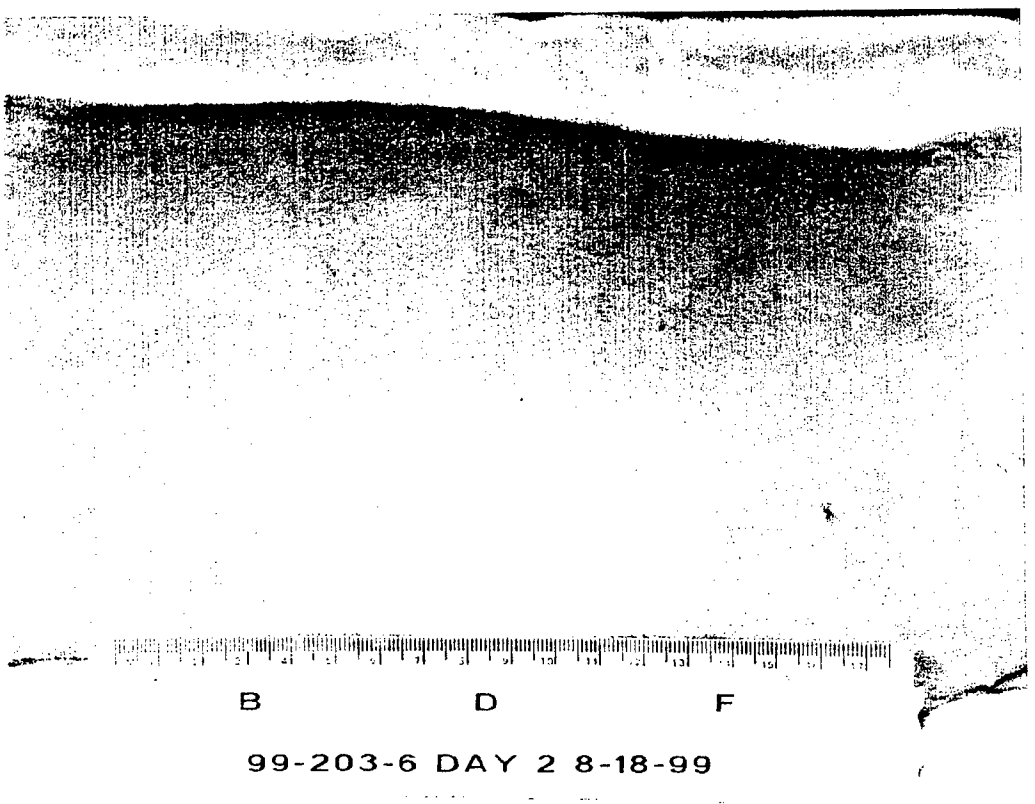


Figure L-13: 60-2B-1: Low Magnification Photomicrograph of Epidermis, Dermis, and Subcutaneous Fat Tissue (2 min Exposure)

Epidermis is incompletely necrotic, evidenced at this magnification by irregular cytoplasmic discoloration. Much of the underlying dermis and subcutaneous tissues (bottom left corner of photomicrograph) is unaffected.

Figure L-14: 60-2B-2: Higher Magnification of Epidermis and Superficial Dermis (2 min Exposure)

Epidermis contains numerous necrotic epithelial cells, evidenced by pyknotic nuclei and intensely eosinophilic cytoplasm. Other epithelial cells exhibit perinuclear or cytoplasmic edema (halos or vacuolar spaces), indicating severe injury. Underlying dermis has evidence of vascular congestion.

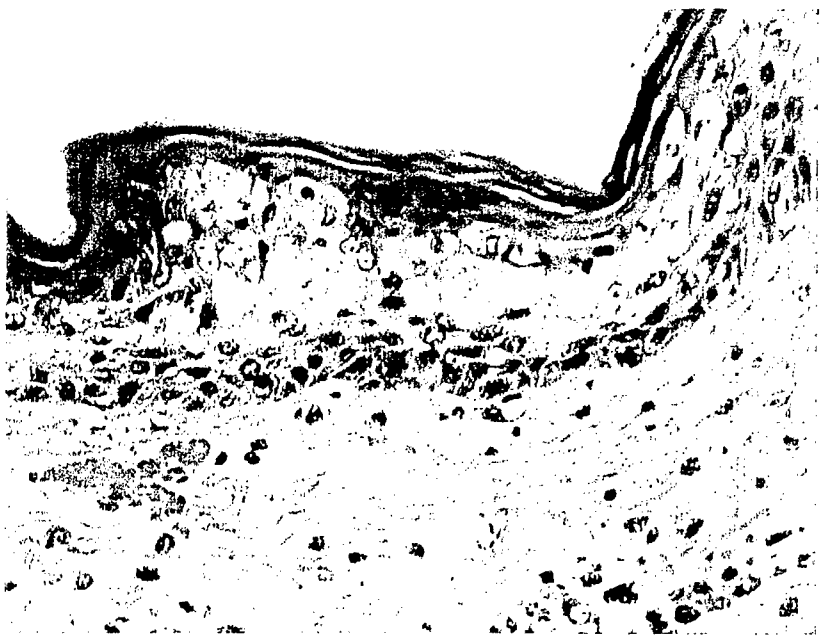


Figure L-15 161-8E-2: Low Magnification Photomicrograph of Epidermis, Dermis, and Superficial Subcuticular Tissue (30 min Exposure)

Epidermis is uniformly necrotic, evidenced at this magnification by the eosinophilic coloration of the cytoplasm. Underlying dermis and subcutaneous tissues (bottom right corner of photomicrograph) are also necrotic and edematous.

Figure L-16 161-8E-1: Photomicrograph of Epidermis and Superficial Dermis (30 min Exposure) .

Epidermis is uniformly necrotic, evidenced by dark, shrunken (pyknotic) nuclei and eosinophilic coloration of the cytoplasm. Underlying dermis also is affected; blood vessels are congested and endothelial necrosis and hemorrhage are present.

Figure L-17 203-6A-1: Low Magnification Photomicrograph of Epidermis, Dermis, and Subcutaneous Fat (0 min Exposure)

All Tissues are normal.

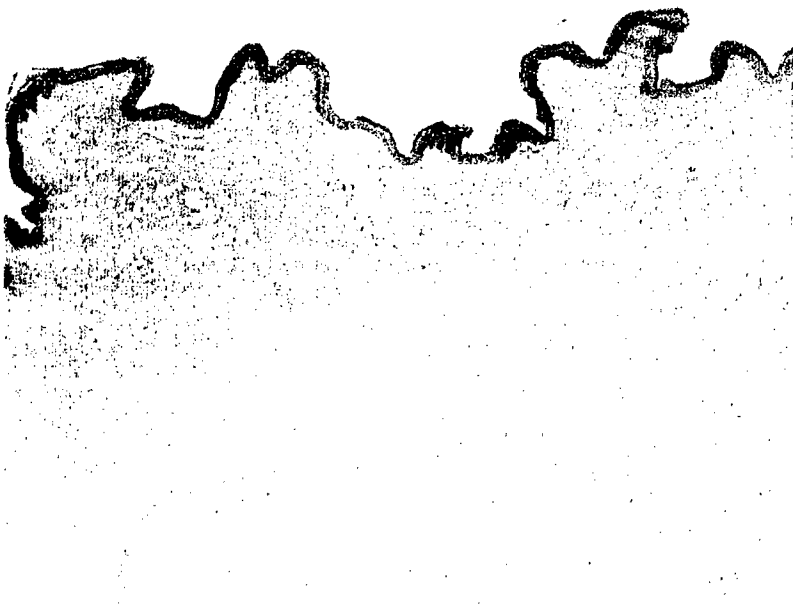
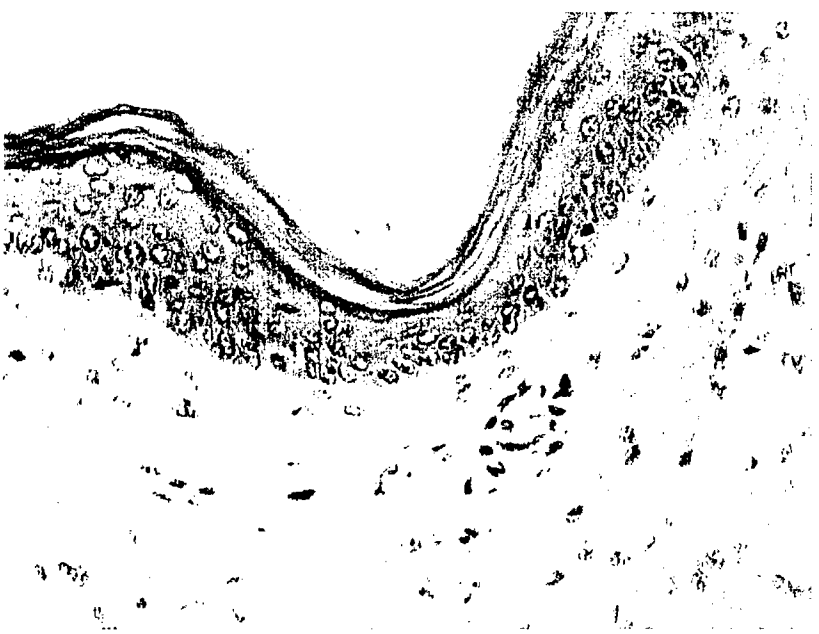


Figure L-18 203-6A-2: Higher Magnification of Epidermis and Superficial Dermis (0 min Exposure)

All tissues are normal. Compare to affected skin sites from animals exposed to agent. Normal nuclei are pale, large and oval; necrotic nuclei (indicative of dead cells in pigs 99-161-8 and 99-60-2) are dark and shrunken.



ATTACHMENT M

Decontamination

Date: May 7, 1996

To: Study File Task33

From: Frances M. Reid

Subject: Meeting for decontamination procedure and proof of decontamination of swine percutaneously exposed to HD.

The following method is my interpretation of the May 7, 1996 meeting with Carl Olson, Tim Hayes, Tom Dreier, Dave Stitcher, and Frances Reid. This procedure of decontamination and proof of decontamination is to be followed for percutaneous dosing of neat HD to create a deep-partial to full-thickness burn. If there are any changes, please ccmail to me in writing by noon on May 8, 1996. This method will be tested on pig 4 of Task 33 and may be modified as needed after consultation with Carl Olson, Tim Hayes, Tom Dreier, Dave Stitcher, and Frances Reid.

TASK 33 DECONTAMINATION PROCEDURE AND PROOF OF DECONTAMINATION

Decontamination Procedure

On each dose site apply:

1. Dry gauze wipe wiped/rubbed over each dose site. Use both sides of gauze wipe and repeat with new gauze wipe.
2. Warm water-soaked gauze wipe rubbed over each dose site, using both sides for 30 seconds per side.
3. Repeat warm water-soaked gauze wipe procedure a total of 12 times, then dry each site (dry gauze wipe).
4. Using an uncontaminated warm water-soaked sport-towel each time, rub dose sites for 15 seconds four times, and follow with a dry sport-towel to dry dose sites.
5. Repeat step 4 an additional 11 times.
6. Allow animal to dry for 30 minutes.

Proof of Decontamination

7. Apply tent, allow tent space to equilibrate for 15 minutes then sample with the Minicams®. If the sample is **at or below 10 TWA**, the animal is removed from the hood and placed in the isolator cage (see discussion below). If the sample is above this TWA level, then the animal will be decontaminated as follows:

    a. 0.5 percent bleach-soaked gauze wipe rubbed over each dose site and applied at 20 seconds per side.
    b. Repeat step 7. a.
    c. Gently rub (rocking motion) each dose site with a warm water-soaked gauze wipes applied at 30 seconds per side.
    d. Repeat step c. an additional 3 times.
    e. Apply 0.5 percent bleach-soaked sport-towel over both dose sites for 30 seconds.

f. Apply warm water-soaked sport-towel over both dose sites for 30 seconds.
g. Repeat step f. for a total of 4 times.
h. Dry dose sites and allow to dry for 20 minutes before applying tent.
i. Re-sample for Minicams®.

ANIMAL ISOLATOR CAGING

Assuming that the air flow in the hood with sashes down can draw enough air to maintain adequate air exchanges within the isolator for an animal, the following method to maintain an animal until proof of decontamination will be tested.

The isolator cage is in room 7 (dosing room) located at the opposite-end of the hood bank of 7 south. A hose is run from the rear exhaust hose-connection into the 7-south hood bank. This connection is fitted with a flow meter to check air flow from the isolator into hood. If air flow is found to be too high for an animal when tested then the isolator caging will need to be adjusted to regulate air flow.

Two people, wearing butyl aprons over scrub suits, respirator connected to main tank, and clean latex/nitrile gloves placed over clean butyl gloves, will remove the animal from the sling and place it in the cage. All other personnel will leave the room. Should an additional person be needed to assist them, they must be clothed as above. The cage is opened and prepared for the animal prior to its removal. When proof of decontamination is **at or below 10 TWA** then the animal is untied from the dosing sling, the anesthesia turned off, the cuff deflated, and the tape securing the endotracheal tube to maxilla is cut. The animal is removed from the sling, placed in the isolator cage, and the endotracheal tube gently removed. Once the isolator cage is closed and secured, respirators may be removed and personnel may return to the room. The animal will remain in this cage until proof of decontamination. Feed and water may be provided.

Proof of decontamination will be attempted at least 24 hours after decontamination and requires tenting of the dosing sites for 15 minutes equilibration and sampling using the minicams. To remove the animal from the isolator, the sample must be **at or below 0.5 TWA**. The animal in the isolator is sedated (half to three quarters of the dose of telazol/xylazine combination is recommended) and may be placed in the sling or on a tie-down board in the hood in room 7. Personnel handling the animal until proven decontaminated will be dressed as described above. Other personnel will not be in the room until the animal is safely in the hood or in the isolator.

JWR 5-7-96

Reid, Frances

From: Graham John S [John.Graham@amedd.army.mil]
Sent: Thursday, May 06, 1999 2:48 PM
To: 'Reid, Dr. Frances'; 'Waugh, Jack' JWR 5-6-00
Cc: 'Stotts, LTC Richard'
Subject: weanling pig off-gassing

Dr. Reid and Mr. Waugh,

I have a request to modify the decon procedure you use after dosing the weanling pigs with percutaneous liquid HD on Task 94-33. The procedure you follow now is to perform a dry dab with a masslin sports towel for 30 seconds, followed by 2 wet dabs (30 seconds then 60 seconds). I request that the wet dabs be eliminated, leaving just the dry dab.

In our weanling pig model for TSP screens here at ICD, we found that wiping off the TSPs after agent exposure with water exacerbates the resultant lesion significantly. We were able to show statistically that when you expose the experimental sites to water following HD vapor exposure (with or without TSPs), the lesions were significantly worse, and out of range of data in our historical database. We believe this is because the water is being absorbed by the stratum corneum and is activating the HD, rather than hydrolyzing the agent (which would actually require a larger volume of water and vigorous rubbing). We have therefore stopped the use of washing off the TSPs with water following use of a dry swab.

With these results in mind, I was concerned that we were similarly exacerbating the liquid lesions in the pig model used in Task 94-33. This will likely play an important role in our upcoming range-finding experiment on our quest to generate superficial dermal burns. The use of water as a "decon" will make the lesions worse. As we can't control the amount of water absorbed by the stratum corneum from pig to pig, I am concerned its use will interfere with our interpretation of the results, and our ultimate success of the study.

In an attempt to ascertain if NOT using water increased the length of off-gassing (currently around 24-30 hours at our facility), I recently exposed some weanling pigs to HD liquid as prescribed by our/your wound healing model with only a dry swab for decon. I then checked their off-gassing time using a Minicams. I then evaluated the results statistically by 3 different methods, all yielding the same results. I performed a Dixon's Q test for outliers, made a box plot, and had our biostatistician run a Z test. We found no statistically significant differences in the off-gassing times of these no-water pigs compared to historical data where water was used.

Dr. Braue and I do not feel that the use of water as a decon is warranted, that it is perfectly safe to omit the wet dabbing, and that it will not increase the time it takes for the animals to stop off-gassing (e.g., the animals won't be moved out of engineering controls back into their pens any later than they already are).

If you have further questions, please consult with Dr. Braue at (410) 436-2848, or wait until I am out there next week. I will bring the statistical analyses out with me for your perusal. Should the need arise, I will be more than happy to sit down with the two of you, Mr. Stitcher and Dr. Estep to share our results and resolve any safety issues. Thanks, and

have a good weekend!

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